RESPIRATORY METABOLISM IN SOME TREMATODES

ABSTRACT

(Ph. D. THESIS)

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ABSTRACT

Studies on the respiratory metabolism of parasites have been confined largely to protozoans, nematodes and cestodes, and trematodes have remained somewhat neglected. However, very little work has been done in this field of trematode physiology on a comparative basis. In Aligarh, cattle and fish harbour various species of trematodes in enormous numbers. These trematodes occur in different habitats and provide a favourable opportunity to study some of the comparative aspects of the respiratory metabolism from the point of view of interspecific differences, similarities or differences in trematode respiration which may exist as a result of inhabiting oxygen rich and oxygen poor environments, and also inhabiting poikilothermic and homeothermic definitive hosts. For this study the author selected four species of trematodes: Isoparorchis hypselobagri, from the swim bladder of the catfish, Wallago attu: Cotylophoron cotylophorum, Gastrothylax crumenifer from the rumen and Gigantocotyle explanatum from the liver of the Indian water buffalo, Bubalus bubalis. These species were studied mainly with respect to the effect of temperature, pH, substrates, chemicals, hormones, ions, osmotic stress, carbon monoxide, anaerobic incubation and various substrates of the TCA cycle on the respiration of

these trematodes.

I. hymenolobatri consumes more oxygen than the mammalian trematodes. Among the mammalian trematodes, G. explanatum consumes more oxygen than the rumen trematodes, while among the rumen trematodes, G. crumenifer consumes more oxygen than the C. cotylophorum. Such differences in the normal oxygen consumption are probably due to the water content and dry weight of the trematode, high and low oxygen tensions in their respective habitats and also due to species differences.

The optimum temperature for oxygen consumption is different in the fish and the mammalian trematodes, i.e., 30° and 40°C respectively. In the fish trematode, the high temperatures ($< 40^{\circ}C$) are more deleterious than the lower temperatures while in the mammalian trematodes, lower temperature causes greater retardation in their oxygen consumption and higher temperatures are not as deleterious in this case as they are in the case of the fish trematodes. Such differences are probably due to the fact that the former trematodes live in poikilothermic and the latter trematodes inhabit homeothermic animals. The results of the present investigation reveal that metabolic temperature response of these trematodes closely parallel the body temperature of the definitive host.

The pH has pronounced effect on the QO_2 of these trematodes. There is an optimum range over which the QO_2 of the

fish, rumen and liver trematodes remain more or less unaltered. Present investigation reveals that QO_2 of parasite depends upon pH of the microenvironment in which these parasites live and support the fact that the nature of the habitat has influenced biochemical and physiological characteristics of the parasites living in that habitat.

Various substrates including hexoses, disaccharides, pentose sugar, aminoacids, glycerol and α GPA cause a significant increase in the respiratory rate of these trematodes and the extent of stimulation of oxygen consumption is different with different species. Maltose was not at all utilized by any species. Among various substrates used, glycerol and glucose were found to be more stimulatory in the mammalian and the fish trematodes respectively. If oxygen consumption is considered as a parameter of hexose utilization, than glucose and fructose were found to be more stimulatory and easily utilized than amino acids in all the four species of trematodes. It is evident from the present results that trematodes make use of carbohydrates, amino acids and other substrates but they exhibit species differences and are adapted to utilizing one substrate better than others. The results of the present investigation suggest that these substrates are readily taken up and utilized as energy source in the metabolism of trematodes, and also the trematodes have food preference.

Several chemicals were tested for their inhibitory or stimulatory effect on the respiration of these trematodes. Among all the chemicals tested, 2,4-dinitrophenol and 2,4-dinitrocyclopentyl phenol were found to stimulate the oxygen uptake, whereas KCN and diethyldithiocarbamate were found to be most and least inhibitors of respiration respectively in all the four species under study.

The degree of stimulation or inhibition of oxygen consumption caused by various chemicals is probably dependent upon the differential permeability of the tegument which appears to be under the influence of parasitism in different habitats. Such studies provide an indirect evidence about the different metabolic pathways in trematodes and also will be helpful in understanding the host-parasite relationship and for the chemotherapeutic studies.

Thyroxin and 5-HT have a stimulatory effect, histamine and adrenaline have depressing effect on the respiration of trematodes, while noradrenaline, testosteron, and progesteron have statistically insignificant effect. The extent of inhibition or stimulation is more or less of the same order with the exception of insulin in the case of G. explanatum, where oxygen consumption increases in the presence of insulin. It is probably due to the fact that this parasite of liver may be more sensitive

to the action of insulin.

It is concluded that direct participation of host hormones as metabolic regulators in the control of the metabolism of the parasite may form a basis for the symbiotic relationship between host and parasite.

It was noticed that Na^+, K^+, Ca^{++}, Mg^+ and PO_4 ions have stimulatory effect on the respiration, although the degree of stimulation by various ions is different. In all the three species under study K^+ has maximum while Na^+ has least stimulatory effect on the respiration. The degree of stimulation of various ions on the QO_2 can be expressed as follows:

I. hypselobagri	$Na = Mg < Ca < PO_4 < K$
G. crumenifer	$Na < Ca < Mg < PO_4 < K$
G. explanatum	$Na < Ca = Mg < PO_4 < K$

It can be concluded from the present investigations that Na^+, K^+, Ca^+, Mg^{++} and PO_4 are important ingredients of the saline which are required for the optimum respiration of the trematodes. Such studies will be fruitful in devising new culture media.

The results of the osmotic studies clearly show that both hypotonic and extreme hypertonic salines are inimical to the oxygen uptake in all the three species under study. However, cat-

fish trematode is less sensitive hypotonicities than extreme hypertonicities. The mammalian species are equally sensitive to hypo- and hypertonic media and the extent of depression in oxygen consumption is similar in both species.

Carbon monoxide causes depressing effect more in I. hyposelobagri than in the mammalian trematodes. The maximum inhibition in the fish trematode is probably due to the fact that I. hyposelobagri lives in an oxygen rich environment, whereas other trematodes under study live in oxygen poor habitats. The degree of inhibition is directly proportional to the incubation time of the trematodes with carbon monoxide. It can be concluded from the present study that trematodes also to some extent can be considered to be facultatively aerobic animals; trematode haemoglobin may be playing some role in oxygen storage or transport, and a cytochrome system might be functional in some of these animals.

Anaerobic incubation of I. hyposelobagri develops oxygen debt which results in a respiratory overshoot when exposed to air and as the anoxic incubation time increases so does the respiratory overshoot. The present study suggests that I. hyposelobagri may possess a mechanism by which it can live both aerobically as well as anaerobically.

Increased oxygen uptake in the presence of various substrates of the TCA cycle provides an indirect evidence in support of an operative TCA cycle in I. hypselobarri.

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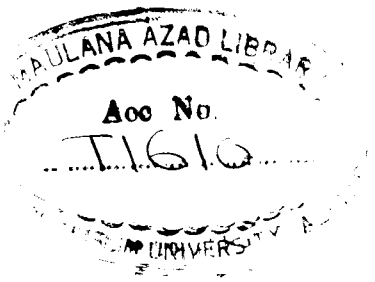

By

WAJIH AHMAD NIZAMI

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This is to certify that the thesis entitled "Respiratory Metabolism in Some Trematodes" which is being submitted by Mr. Wajih Ahmad Nizami, embodies original work done by the candidate himself. The entire work was carried out under my supervision between 1974-1976 and that I allow him to submit the same in fulfilment of the requirements for the degree of Doctor of Philosophy in Zoology of this University.

Ather H. Siddiqi
Supervisor

C O N T E N T S

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C H A P T E R - I

INTRODUCTION

In spite of many advances that have been made in trematode physiology and biochemistry during the last twenty years in various parts of the world, there are still many gaps and lacunae in our knowledge of the subject. In India most of the work so far done in parasitology has been confined mainly to the morphology and taxonomy of various groups of parasites. Only Goil (1953-73) has published some preliminary observations in the field of trematode physiology which has otherwise remained completely neglected in this country. Recently at Aligarh, a Parasitology Research Laboratory has been established, in which various aspects of the trematode physiology and biochemistry are being studied.

Whatever work has been done so far in the field of trematode physiology concerns mainly two species, i.e., Fasciola hepatica and Schistosoma mansoni and only occasionally some other species. Particularly the knowledge of respiratory metabolism of trematodes is still in its infancy. This may be due to the fact that in studying the respiratory metabolism, certain difficulties arise and one has to overcome the same. The trematodes represent a peculiar group of parasites because of the complex nature of their life cycles.

During a trematode life cycle, the larval stages may pass from a free living stage to invertebrates and than finally develop into adults in a vertebrate definitive host. Hence the survival of these parasites is therefore influenced by the general biotic factors associated with the aquatic environment as well as by the intimate physiological and immunological interactions which are a part of any host-parasite relationship. The adult trematodes live in diverse kinds of poorly understood and rarely investigated habitats, and knowledge of the existing pO_2 in these micro-environments is not known except for more than a few of them. Some habitats are such that the estimation of pO_2 requires the use of more modern and sophisticated equipment like oxygen electrode and oxygen analyzers. Further it is still more difficult to determine the oxygen consumption of trematodes while they are still in vivo. Therefore one has to resort to experiments on oxygen uptake of these endoparasites in an in vitro system which involves the removal of the parasite from the host and simulation of conditions in vitro which are as near as possible to those believed to exist in vivo. Removal of a parasite from the habitat disturbs a delicate and intimate balance of host-parasite relationship, because the host provides many important physiological and biochemical needs of the parasite. This then does not permit to study the respiration of these parasites in vitro for long periods.

For the study of respiration, the suitable apparatuses for routine measurements of oxygen uptake of parasites are the Warburg manometers, the polarograph using Clark's oxygen electrode, the Scholander respirometer, and for small trematodes or their larval stages, the Cartesian diver respirometer.

All parasites studied so far under aerobic conditions have been found to consume oxygen and produce carbon dioxide regardless of whether they have primarily aerobic or anaerobic mode of life in their natural habitat. The question whether helminths lead a predominantly aerobic or anaerobic life can be decided only on the basis of knowledge of the oxygen tension of their surroundings, the response to lack of oxygen and the factors determining the respiratory rates. von Brand (1973) classified parasitic habitats into "oxygen free (or perhaps more accurately oxygen poor) environments, unambiguous aerobic habitats and one potential habitat with excessively high oxygen tension, the swim bladder of some fishes."

The concept of aerobic and anaerobic metabolism as applied to parasites need careful examination. It is doubtful whether any parasitic helminth can be made to conform entirely with either category, although some of the parasitic protozoa may be true anaerobes.

The nature of the habitats of parasites is such that, with few exceptions, there will be almost always some oxygen present,

even if in only small quantities. According to Bryant (1971) "... parasites are "metabolic opportunists"; if a resource is available and is useful, then it will be used. Its use need not be confined to respiration alone, for there are many other processes that require oxygen. The parasite assumes, a chamaeleon-like physiological quality -- the type of their metabolism whether aerobic is fitted to the prevailing environmental condition. They must therefore possess some mechanism which will enable them to detect and then react to a change in that environment, the precise nature of this mechanism is one that still mystifies parasitologists". The available information on respiratory metabolism (von Brand, 1973) suggests that adult parasitic helminths are facultative aerobes and, although they show a marked tolerance to lack of oxygen they are able to utilize it when available. The evidence comes from measurements of the rate of glycolysis and the detection of enzymes of glycolysis and the Krebs cycle.

The trematodes have been reported to possess two types of respiratory pigments: haemoglobin and cytochromes, both haem containing compounds. Though occurrence of haemoglobin in the tissues has been reported in a number of trematodes, nothing is known about its origin or functional importance. According to Pantelouris (1967) they may be functionless by products of the nutrition of worms whilst Freeman (1963) thinks that the pigment may simply

facilitate oxygen diffusion. Lee and Smith (1965) have pointed out that endoparasitic haemoglobins have such a high affinity for oxygen that they are completely saturated at oxygen tensions as low as 1 or 2 mmHg. Since these oxygen tensions are within the range normally encountered by the parasites, it appears that the chief function of the worm haemoglobin is facilitation of oxygen transfer rather than of oxygen storage. However, carbonic anhydrase has also been reported from the large liver fluke, which indicates that the worm haemoglobin might be useful in the transport of carbon dioxide.

The continuous supply of energy in trematodes, to motivate the complex synthetic processes associated with growth and development and to support such physical activities as feeding, excretion, reproduction etc., is obtained by the generation of energy rich phosphate bonds in the form of ATP. This is usually associated with the oxidation and reduction of the carbohydrates, although protein or lipid may in some instances be the initial source of energy. This degradation of the fuel involves the uptake and utilization of oxygen. Data on the metabolic pathways are fragmentary in trematodes, but there is some evidence that glycolysis and tricarboxylic acid cycle are operative. Also, cytochromes have been reported from some trematodes, but glycolysis, TCA cycle and cytochrome systems are not completely identical with those occurring in

an aerobic tissue though many enzymes of these pathways have been demonstrated in trematodes.

The knowledge of the respiratory metabolism of trematodes is significant in many ways. Firstly, it makes an important contribution to the study of comparative invertebrate biochemistry and provides an approach for the understanding of biochemical aspects of parasitism. The parasitic mode of life is one of the most interesting and complex phenomena of animal associations, which has brought about not only morphological and physiological but also many biochemical adaptations in parasites. Secondly, knowledge of respiration is an important ingredient in the rational development of chemotherapy of trematode infections in man and domestic animals. At present, the recent trend of research in chemical and veterinary helminthology is to develop such anthelminthic drugs which do not cause any harm to the host but are effective against the parasites. Today pharmacologists are explaining drug action on a physiochemical basis. The basic concept is that the drugs exert their effect by interfering with some metabolic activity of parasites to cause membrane alteration, enzyme inhibition or uncoupling of energy mechanism. More effective eradication of trematode parasites can be achieved only when physiological and biochemical information about the parasites and their relationship to the host is available. Therefore, for developing really effective drugs, a clear understanding of the physiological and biochemical aspects of trematode parasites is necessary.

C H A P T E R - II

HISTORICAL REVIEW

At the beginning of this century Weinland (1901) pointed out that intestinal worms like Ascaris were not capable of consuming oxygen and the possibility of aerobic processes was conceded only for the eggs. Weinland's concept was accepted for the next 30 years until it was shown by Daniel (1931), Alt and Tischer (1931) and Adam (1932) that the intestinal parasites were capable of consuming oxygen. During the last twenty years many more helminth parasites have been studied and which have been found to consume oxygen.

All stages in the life cycle of the digenetic trematodes have been found to utilize oxygen when it is available. Vernberg (1963) and von Brand (1973) reviewed the available information and concluded that the adult trematodes are facultative aerobes, and according to Goil (1958), Bueding (1949) and von Brand (1952) some of them are quite tolerant to lack of oxygen. The available data on oxygen consumption indicates that respiratory rate of trematodes is dependent (i.e., directly proportional) on the external oxygen tension and other factors as pointed out by Harnisch (1932), van Grembergen (1949), Mansour (1959), Bueding

(1949, 1950), Vernberg and Hunter (1961), Vernberg (1963), and Bruce et al. (1971).

The type of metabolism of a parasite depends on a number of factors including the presence or absence of oxygen in the habitat and on the parasite's ability to bind oxygen with its respiratory pigment. Most of the parasites live in environments with high oxygen tension, for example, parasites of vertebrate lungs are exposed almost to the same oxygen tension as the free living animals. Another habitat rich in oxygen is blood. The data on the amount of oxygen present in a wide variety of habitats have been collected and discussed by von Brand (1946), who later summarized this information especially in relation to the oxygen available to internal parasites (von Brand 1952). Read (1950) has reviewed the information on the oxygen tension in the small intestine. According to Rogers (1949), the oxygen tension in the small intestine of rat ranges from 8.9 to 30.2 mmHg and depends upon the distance from the pylorus. This pO_2 may be biologically significant. On the other hand Chaigneau and Charlet-Lery (1957) observed that the intestine of large vertebrates is either poor in oxygen or is sometime completely oxygen free. The best known anaerobic or at least near anaerobic habitat is the bile duct (von Brand and Weise, 1932), and the rumen of various ruminants (literature in Levitt, 1970). The excessively high

oxygen tension (as high as 98%) has been found in the swim bladder of fishes (Scholander and van Dam, 1953), and may exceed 100 atm (Wittenberg 1958) in the swim bladder of deep sea fishes. According to Morkov (1961) "oxygen is present in the blood of fishes. For example the pO_2 in venous blood is between 2 to 11 mmHg. The metabolism of the blood parasite of fishes (e.g., the trematode Sanguinicola) is probably of the oxidative type, although no assumption can be made in this respect. For example contrary to expectation, most of the reactions of the blood flukes Schistosoma haematobium, proved to be anoxybiotic (Bueding 1949)."

According to Puchkov (1954), the average oxygen content in the swim bladder of fresh water fishes like Perca and Esox is up to 19-25%. Similarly, Siddiqi and Nizami (1975) reported that the swim bladder of Wallago attu contains sufficient quantity of oxygen ranging from 22.0 to 58.0 mmHg, carbon dioxide 1.59 to 10.48 mmHg and nitrogen as the major gas component. The presence or absence of the swim bladder trematode, Isoparorchis hypselobagri did not influence the oxygen or CO_2 content of the swim bladder gas.

Most parasites tolerate lack of oxygen for shorter or longer periods but their sensitivity to oxygen poisoning and their oxygen requirements for prolonged survival vary from species

to species. A survey of literature shows that trematodes are some time very resistant to lack of oxygen. Hobson (1948) has justifiably pointed out that all data on aerobic survival are derived from experiments carried out at the oxygen tension of atmospheric air, while unquestionably the endoparasitic forms usually encounter much lower oxygen tensions in nature. It has been observed that in vitro survival of trematode parasites under aerobic and anaerobic conditions varies markedly from species to species. Ross and Bueding (1950) have shown that Schistosoma mansoni survives only five days under anaerobic while 12 days under aerobic conditions; whereas Rohrbacher (1957) observed that Fasciola hepatica survived 13 days under aerobic conditions and 20 days under anaerobic conditions. Erhardt (1939) found that Opisthorchis felineus survive 18 days both under aerobic and anaerobic conditions. Nizami and Siddiqi (1975) found that Isoparorchis hypselobagri, a parasite of the swim bladder of the catfish survived in vitro for 30 days under anaerobic and 49 days under aerobic conditions.

The foregoing review of literature indicates that the parasites from various habitats in different animals encounter various tensions of pO_2, but irrespective of their in vivo conditions, they consume oxygen under in vitro conditions.

I. Oxygen Consumption

All stages in the life of the digenetic trematodes have been found to utilize oxygen when available. The oxygen consumption in trematodes have been studied by a number of workers which shows that it varies from species to species. So far the following digenetic trematode parasites have been studied. Fasciola hepatica (van Grenbergen, 1949); Schistosoma mansoni (Bueding, 1950); S. japonicum (Shimomura, 1959); Clonorchis sinensis (Nagamoto and Okabe 1957); Dicrocoelium dendriticum (Eckert and Lehner, 1971); Echinostoma revolutum (Taft and Fried, 1968), Paragonimus westermani (Shimomura, 1959); P. ohirai (Bruce et al., 1971); Paramphistomum cervi (Lazarus, 1950); Gynaecotyle adunca (Vernberg and Hunter, 1959); Himasthla quissetensis (Vernberg and Hunter 1963); Saccocoelium beauforti (Vernberg and Hunter 1961); Fasciola gigantica (Goil 1961); Gastrothylax crumenifer and Paramphistomum explanatum (Goil, 1958); Pleurogonimus malaclemyes (Vernberg and Hunter 1961) and Isoparorchis hypselobagri (Siddiqi and Nizami, 1975). All the species mentioned above consume oxygen in vivo whether they live in oxygen poor or oxygen rich environment.

II. Effect of Temperature

Though the influence of temperature on metabolic rate has been one of the most widely studied relationships in the whole

domain of respiratory physiology, the metabolic temperature response of trematodes have been studied in a few species. The trematodes experience extremes of temperature during their life cycle. The larval stages develop in poiklothermic hosts followed by a free living cercarial stage, which may pass through a poiklothermic second intermediate host (if present) and then mature in either a homeothermic or a poikilothermic vertebrate definitive host. According to Vernberg and Hunter (1961) the metabolic temperature response of adult trematodes is closely correlated to the body temperature of the definitive host. Vernberg and Hunter (1961) made a comparative study of three species of trematodes from three different hosts, Saccocoelium beauforti from fish, Pleurogonimus malaclemys from turtle, and Gynaecotyle adunca from bird, and found that the fish parasite showed an increased respiration rate as temperature was raised up to 30°C, and at 36°C the rate was decreased. The turtle parasite, which is subjected to a greater temperature variation than the fish parasite, showed an increase in respiratory rate up to 36°C, while the bird parasite showed an increase up to 41°C.

While studying the effect of temperature on the respiration of larval trematodes, Vernberg (1961) found that the response of larval stages do not necessarily reflect the body

temperature of their host, because the larvae of the fish parasite die within half an hour at 39°C, while the larvae of the bird parasite remain alive and active at the end of the six hour period at 41°C.

III. Effect of pH

The effect of pH on the respiration of trematode has been studied by Bueding (1950) in Schistosoma mansoni while oxygen consumption remains unaltered from 6.8 to 8.9, but below or above this range of pH the oxygen uptake of the parasite is adversely affected.

IV. Effect of various substrates

The effect of various carbohydrates and amino acids on the respiration of adult trematodes have been studied in only a few species. van Grembergen (1949) found that glucose and alanine had no influence but α-glycerophosphoric acid and glutamic acid had a strong stimulatory effect on the oxygen uptake of Fasciola hepatica. Mansour (1959) suggested that in presence of oxygen the glucose utilization slightly decreases in F. hepatica, while Bueding (1950) found that glucose had a stimulatory effect on the respiration of Schistosoma mansoni. Read and Yogore (1955) reported a QO_2 value of 0.74-0.86 for Paragonimus westermani in Krebs-Ringer phosphate buffer containing

0.01 M glucose while Shimomura (1959) reported a QO_2 value of 2.8. Taft and Fried (1968) found that oxygen consumption of 8-28 days old Echinostoma revolutum was 2.8-4.4 $ul O_2$/mg dry wt/hr in the absence of glucose, while in the presence of glucose, 7-28 days old worms consumed oxygen at the rate of 3.0-4.5 $ul O_2$/mg dry wt/hr. Vernberg and Hunter (1963) found that proline and glutamic acid were slightly effective in increasing the respiration rate, while glucose and mannose proved to be most effective in increasing the respiration rate of Himasthla quissetensis. Bruce et al. (1971) measured the respiration of Paragonimus ohirai 25 hr after collection, and found that when glucose was added to the medium, the respiration rate was increased while one hour after collection the respiration rate was decreased. They also found that glucose, glucosamine, maltose, fructose, lactose, mannitol, mannose, and galactose produced a significant increase in QO_2 after 25 hrs of collection of P. ohirai. Contrary to these findings, Eckert and Lehner (1971) found that glycerol increased the rate of oxygen uptake, whereas glucose, galactose and fructose produced no stimulatory effect in Dicrocoelium dendriticum. They found that glycerol was absorbed in larger amounts than glucose, and substrate absorption was slightly higher under aerobic than under anaerobic conditions. Recently Siddiqi and Nizami (1975) found that when glucose is added to the medium, the oxygen consumption

was increased 50% in the case of Isoparorchis hypseloba ri, which suggests that glucose is readily taken up and utilized as an energy source.

. Effect of Chemicals

Th' is one aspect of trematode respiratory physiology that needs immediate attention of parasitologists. Most of the literature on the subject has been reviewed by van Grenbergen (1949), Jones (1966), Frayha (1972), von Brand (1973) and Gibson (1975). van Grenbergen (1949) found that malonic acid, ethyl urethane and KCN have inhibitory effect, while 2,4-dinitrocyclopentyl phenol (10^{-5}M) and 2,4-dinitrophenol (10^{-5} to 10^{-4}M) have stimulatory effect on the respiration rate of F. hepatica; whereas Coles (1972) reported that in the absence of APD the respiration of _. mansoni was stimulated by 2:4 dinitrophenol (2×10^{-4}M). Prichard and Schofield (1971) reported that azide (10^{-3}M) causes 35% inhibition in oxygen uptake in F. hepatica whereas in S. mansoni, azide causes 53% inhibition in oxygen uptake (Magzoub et al., 1971). KCN was found to be an effective inhibitor of the respiration of S. mansoni (Ross and Bueding 1950; Bueding and Charms, 1951; Magzoub et al., 1971; Coles, 1972). On the other hand, Lazarus (1950) reported that cyanide caused a pronounced stimulatory effect in the oxygen consumption of Paramphistomum cervi.

Nagamoto and Okabe (1959) studied respiration of Clonorchis sinensis and found that 0.03% resochin and stibnal (sodium antimony tartarate) inhibit the gasseous metabolism. Shimomura (1959) observed that in Schistosoma japonicum the respiration was obstructed powerfully by 0.02% potassium antimony tartrate, less powerfully by 0.02% stibnal and only slightly by 0.02% stimon.

Eckert and Lehner (1971) studied the effect of some of the inhibitors of the respiratory chain, and when 2-thionyltrifuro acetone or 8,hydroxyquinoline or salicylaldoxime or KCN or antimycin A is added to the medium, distinct inhibition of oxygen consumption in Dicrocoelium dendriticum takes place. However, the rate of respiration was increased by 2,4-dinitrophenol due to the uncoupling of oxidative phosphorylation. Vernberg and Hunter (1960) found that when malonate, which competitively inhibits succinic dehydrogenase was added to the medium, there was a highly significant decrease in oxygen consumption in Gynaecotyle adunca. In the same parasite, the copper inhibitors (diethyldithiocarbamate, salicylaldoxime, and phenylthiourea) also inhibit respiration. Coles (1973) exhaustively reviewed the effect of various inhibitors on the respiration of S. mansoni. Bueding (1950) reported that arsenite and p-chloromercuric benzoate inhibit the oxygen uptake and this inhibition is not prevented or reversed by an excess of glutathione or of thioglycollate; whereas fluoride and fuadin also

cause inhibition in the respiration of S. mansoni. On the other hand, an inhibitory effect of antimycin A (10^{-6}M), amytal (10^{-3}M) and rotenone (10^{-6}M) in the respiration of S. mansoni was reported by Coles (1972), who concluded that the worms possessed a coupled functional cytochrome system, whereas fluoroacetate partially inhibited oxygen consumption (Coles, 1970). In another study, Hamajima (1973) found that bithinol stimulated lactic acid production and inhibited the oxygen consumption of adult Paragonimus westermani in vitro.

VI. Effect of Hormones

Though different aspects of the host-parasite relationship of trematodes have been studied by a number of workers, the effect of the secretions of the host on the metabolic activity of these parasites has remained somewhat neglected. Solomon's (1969) comprehensive review of an host endocrine-parasite interaction emphasized the paucity of any suitable conclusions. Various effects of thyroxine and thiouracil (Dobson, 1966a) and progesteron (Dobson, 1966b) have been described on the size and intensity of infection. In some nematodes, larval development in the host was found to be influenced by cortisol hormone (Dunsmore, 1961; James and Johnstone, 1967), prolactin, hydrocortisone and oxytocin (Oshima, 1961). Testosteron and estrogen was found to effect the intensity of nematode infection (Katz, 1963).

Rogers and Head (1972) found that noradrenaline was increased by 2-9 times in Haemochus contortus, when infective juveniles were stimulated to develop in vitro by incubation in bicarbonate-carbon dioxide buffer, and he also suggested that this hormone may activate adenyl cyclase system to supply energy for development of the parasite. Frayha et al. (1971) suggested that sex of the host and its hormones play an important role in the development of infection of Echinococcus granulosus.

From the review of the data available, there seems little doubt that the sex of the host influences the metabolism of platyhelminth parasites (Aldrich et al. 1954; Daugherty, 1956). Berg (1957) reported that testosteron altered the expected sex ratio in Schistosoma mansoni, while Robinson (1959) was unable to demonstrate consistent effect of male sex hormones in the same host-parasite combination. Equivocal results were obtained with testosteron by Moor et al. (1954). Most of the effect of the host hormone on the metabolic activity of the parasite comes from in vitro studies. Serotonin (5HT) has been found to occur in microquantities in F. hepatica (Mansour, 1957) and in Aspicularis tetraptera (Anya, 1973). The latter author suggested that serotonin is both neurogenic as well as myogenic in distribution. In F. hepatica homogenates, glycolysis is stimulated by serotonin (Mansour, 1962). In an exhaustive study, Mansour (1957,a,b, 1958, 1959, 1962) suggested that serotonin has stimula-

ting effect on the rhythmical movement, glucose uptake, glycolysis and increase in the phosphofructokinase activity in F. hepatica. Bennett and Bueding (1973) reported a high concentration of 5-HT in S. mansoni.

According to Pantelouris (1964) and Hines (1969), insulin causes marked depletion in the glycogen content of the liver fluke. Sekardi (1966) reported enhanced glucose uptake by F. hepatica when incubated with insulin. Isseroff and Read (1968) do not believe that insulin has any effect on carbohydrate utilization in F. hepatica. In vitro studies employing tri-iodothyronine (Pantelouris, 1965) and thyroxine (Hutton et al., 1972) have failed to show any positive effect of these compounds on intact worms or homogenates of F. hepatica. However, the findings of Cornford (1970) concerning the effect of thyroxine on Schistosomatium douthitti metabolism were confirmed by Abdel Wahab et al. (1971). Jenkins (1961) found that thyroxine had no effect on oxygen consumption of Dugesia dorotocephala. Williams (1968) suggested that this hormone does not directly affect vertebrates and has no effect in invertebrates comparable to those seen in vertebrates. (Prosser and Brown, 1961). Recently Cornford (1974) reported that thyroxine treated Schistosomatium douthitti showed increase tetrazolium reductase and cytochrome oxidase activities, and also increases oxygen uptake in adult as well as in cercariae of this species. Chou et al. (1972) assayed

biogenic amines of P. ohirai and high concentration of 5HT were found in S. mansoni, S. japonicum and S. haematobium. Dopamine was present in S. japonicum and noradrenaline in S. japonicum and S. mansoni. Briggs (1972) studied metabolism of steroid hormones in schistosomes, and suggested that reproductive physiology of the blood flukes may be controlled by steroid hormones. An intensive examination of the effects of various hormones on liver fluke's endproducts (carbon dioxide production, lipid and glycogen) indicate that thyroxine, histamine, epinephrine, norepinephrine, progesteron, testosteron, and hydrocortisone have no significant effect while 5-HT has significant effect on the trematode metabolism.

The importance of histamine has been studied extensively in vertebrates, however, it is still uncertain as to what extent histamine is concerned in affecting any physiological process in trematodes. Mettrick and Telford (1963a, 1963b) reported histamine content from two species of trematodes, Fasciola hepatica and Mesocoelium monodi.

VII. Effect of various Ions

Most of the studies on the effect of ions on the respiration rate of flat worms have been made on fresh water planaria, Dugesia dorotocephala, in which distilled water has a depressing effect (Hess, 1930, Buchanan, 1931), and calcium chloride causes

an initial rise in the metabolic rate; sodium chloride has no effect but potassium chloride causes an immediate rise in oxygen uptake (Hess, 1930). There are, however, fewer studies on the effect of ions on trematode respiration. According to Bueding (1950) the ionic composition of the medium has a marked influence on the respiratory rate of Schistosoma mansoni, especially potassium had stimulatory effect. Phosphates stimulate the respiration of S. mansoni (Bueding et al., 1947), but in scolices of Echinococcus it had an opposite effect (Agosin et al., 1957). In adult Himasthla quissentensis the respiratory rate was depressed at salinities above 10% while in its cercaria no effect was seen (Vernberg, unpublished results).

VIII. Effect of Osmotic Stress

Schlieper (1929), Schwabe (1933) and Flemister and Flemister (1951) demonstrated that crabs consume more oxygen when subjected to osmotic embarrassment, and Krogh (1939), Potts (1954) and Gross (1957) feel that muscular and other activities account for this increase in oxygen uptake and muscular and other activities affect osmoregulation indirectly. Ramamurthi (1968) while studying oxygen consumption of leech under osmotic stress concluded that oxygen uptake was probably related to both osmotic work load and ionic regulation. Such type of study in trematodes has never been made before, though, osmotic and ionic behavior

has been studied extensively by Siddiqi and Lutz (1966) and Siddiqi *et al.* (1975). These authors concluded that all species of trematodes behave as leaky osmometers and loss of Na^+ and K^+ take place by simple diffusion. The differences in osmotic and ionic behavior among trematodes are due to the differential permeability of their teguments. Bair and Peters (1971) found weight loss and oxygen consumption as valid parameters of osmotic activity in *Haematolechus medioplexus*, however, their data on oxygen consumption of this trematode do not support their claim since differences in oxygen consumption in worms subjected to various concentrations of NaCl do not seem to be significant.

IX. *Effect of Carbon Monoxide*

The occurrence of haemoglobin has been reported in trematodes like *Allassostoma* and *Telorchis* (Wharton, 1941) *Dicrocoelium dendriticum* and *Fasciola hepatica* (van Grembergen, 1949); *Fasciola gigantica*, *Cotylophoron indicum* and *Gastrothylax crumenifer* (Goil, 1959, 1961). Besides these findings, Freeman (1963) and Todd & Ross (1966) also studied trematode haemoglobin. Lee and Smith (1965) reviewed the subject exhaustively. Recently trematode haemoglobin was studied by Halton (1967) and Lutz and Siddiqi (1967). The latter workers demonstrated spectrophotometrically and electrophoretically that haemoglobin of *F. gigantica* is a true porphyrin pigment distinct from that of the host. These authors

for the first time suggested that parasite haemoglobin is endogenous. More recently Cain (1969 a,b,c) also studied various physico-chemical properties of haemoglobin of F. buski and concluded that parasite haemoglobin resembles vertebrate myoglobin structurally. These findings clearly indicate that trematodes possess a respiratory pigment. Besides this a functional but slightly modified cytochrome system also exists in trematodes. With the discovery of haemoglobin and cytochrome system, it is evident that carbon monoxide must have some effect on the respiratory metabolism. As West et al. (1966) pointed out, the poisonous action of CO is due to combining with haemoglobin and myoglobin to form carboxyhaemoglobin and carboxymyoglobin. Carbon monoxide also combines with ferrous cytochrome oxidase and prevents the transfer of hydrogen electrons to oxygen.

The effect of carbon monoxide on the respiratory metabolism of trematodes has been little studied. van Grembergen (1949) reported that the respiration of Fasciola hepatica was considerably inhibited by carbon monoxide. von Brand (1973) reviewed the literature on the inhibition of respiration of parasites and suggested that inhibition by carbon monoxide, if reversed by light indicates iron catalysis or the presence of cytochrome system.

X. Effect of anaerobic incubation

Certain animals respond to lack of oxygen by accumulating an oxygen debt and show a subsequent aerobic recovery period and a temporarily increased rate of oxygen consumption. This phenomenon is known as repayment of oxygen debt, respiratory rebound, or respiratory overshoot. Very little is known about this aspect of trematode physiology, and the few data that are available, are contradictory. The literature on this subject has been reviewed by Rogers (1962) and von Brand (1973). The origin and significance has been discussed by Prosser (1050) and von Brand (1946, 1952). Zimmerman and Berry (1949) have given a theoretical consideration of this matter which, however, has been questioned by Goddard and Meeuse (1950). Respiratory rebound occurs only when end products of the anaerobic metabolism accumulate within the tissue and presumably serve as substrates for the increased oxygen consumption (von Brand, 1973). That is why helminths like Litomosoides carini and Schistosoma mansoni (Lueding, 1949, 1950), Gynaecotyle adunca (Hunter and Vernberg, 1955) and Hippostrongylus brasiliensis (Roberts and Fairbairn, 1965) which do not accumulate an oxygen debt may have the ability of excreting the end products more or less completely. According to von Brand (1973), helminths capable of accumulating an oxygen debt, which increases their post anaerobic oxygen consumption to a varying degree, both in respect to the percent stimulation of the rate as well as to the length of

time during which abnormally high values are found. Among helminths the oxygen debt has been reported in Ascaris lumbricoides (Laser, 1944), Strongyloides ratti (Barrett, 1969), Eustrongyloides ignotus (von Brand, 1947), Hymenolepis diminuta (Read, 1956) and Paragonimus westermani (Read and Yogore, 1955). However, Paragonimus ohirai (Bruce et al., 1971) behaves differently. Freshly collected specimens did not accumulate an oxygen debt but when worms were studied after having maintained for 25 hrs in vitro showed a distinctly increased oxygen uptake for at least one hour. Bruce et al. (1971) reported that repayment of oxygen debt did not occur in Clonorchis sinensis.

XI. Effect of Tricarboxylic Acid Cycle Substrates

The tricarboxylic acid cycle has been studied and proved to be a major oxidative pathway in many groups of animals, but whether this is true for parasitic helminths is disputed. The TCA cycle has been studied in a number of nematodes and cestodes but trematodes have remained somewhat neglected and only a few studies are available, which do not allow any generalization. Various intermediate compounds of the TCA cycle have been found to be utilized by trematodes. In Fasciola hepatica citric, succinic, fumaric and malic acids have been demonstrated by Pennoit-De Cooman and van Grenbergen (1942), and van Grenbergen (1949). Bryant and Smith (1963) suggested that there is evidence of a complete TCA cycle in the adult F. hepatica. Sturm et al.

(1969) compared the enzyme activities of F. hepatica with that of bovine liver and found that the metabolism differs from that in the liver and reversal of citrate cycle leads to the formation of propionate as the main end product. Sturm et al. (1972) suggested that enzyme activities of Dicrocoelium dendriticum show a great similarity to those of liver of the host. Citrate, aconitate, a-ketoglutarate, succinate, fumarate, malate and oxaloacetate have been demonstrated in F. hepatica by Bryant and Smith (1963). Thorsell (1963) also was able to detect by chromatography small amounts of intermediates of Krebs cycle: citric (possibly including isocitric), succinic, fumaric and malic acids. She was uncertain about aconitic acid and found only minimal amounts of ketoglutaric acid and oxaloacetic acid in F. hepatica. Hammen and Lum (1962) reported that Entobdella bumpusi, metabolized citric, isocitric, a-ketoglutaric, succinic, fumaric and malic acids. Hamajima (1972) found that the substrates of TCA cycle so far tested generally stimulated oxygen uptake. The highest and the lowest oxygen consumption rate was observed in the adult. Succinate, -ketoglutarate, isocitrate and malate accelerated the oxygen uptake and suggested that the possibility of the presence of a functional TCA cycle in the respiratory metabolism of Paragonimus westermani, P. miyazakii and P. ohirai.

Many enzymes of the citric acid cycle of schistosomes have been demonstrated by various workers: citrate synthase,

isocitrate dehydrogenase (Coles, 1972) succinic oxidase (Smithers et al., 1965; Coles, 1973) and malate dehydrogenase (Condedal Pino et al., 1966; Bueding and Saz, 1968; Coles, 1970, 1971, a,b, 1973). According to Coles (1973) schistosomes possess a functional citric acid cycle. Kohler and Hanselmann (1973) studied the TCA cycle in Dicrocoelium dendriticum, and suggested that a complete sequence of enzymes is present in this worm. When comparing the activity pattern found in the parasitic tissue with the classical Krebs cycle activities of vertebrate tissue, more similarities than differences can be observed. Most obvious difference is seen in the activity level of the -ketoglutarate dehydrogenase complex which is considerably lower in D. dendriticum than in the corresponding rat liver enzyme. Beside these findings, Vernberg and Hunter (1960, 1963) studied citric acid cycle in Gynaecotyle adunca and H. quissetensis. According to Pantelouris (1967) "It cannot be doubted that Krebs cycle reactions are in evidence in the adult liver fluke."

C H A P T E R - III

STATEMENT OF PROBLEM

It is evident from the foregoing review of the literature that the respiratory metabolism of trematodes has been studied in a number of species, but no generalizations concerning respiratory metabolism can be made on the basis of the present status of our knowledge, because various aspects of respiration have been studied in different species. Bueding and Most (1953) suggested that no generalization concerning helminth metabolism can be made on the basis of results obtained with one member of the group, even though they may be related to each other morphologically or taxonomically, thus pointing the necessity of studying each member separately. This is particularly true with trematode's respiration. If one accepts what has been stated above, there is an obvious need to study as many different species as possible occupying similar and different habitats.

In Aligarh, the Indian water buffalo Bubalus bubalis and the catfish, Wallago attu, harbour enormous numbers of various species of parasites. For the present study, four different

species of trematodes were chosen. They are: Cotylophoron cotylophorum, Gastrothylax crumenifer from the rumen, Gigantocotyle explanatum, from the bile duct of the water buffalo, and Isoparorchis hypselobagri from the swim bladder of the catfish, Wallago attu. The choice of these four species was considered important since they parasitize three different habitats in two different species of vertebrate hosts and provide favourable opportunity to study some of the comparative aspects of respiratory metabolism and interspecific differences among species that might exist as a result of living in various habitats in similar and different hosts.

In addition to this, it will provide an insight into the intrinsic physiological relationship between the host and the parasites. The swim bladder, liver and rumen are three different types of habitats which differ from each other in oxygen tension, food content as well as in the osmolarity of the medium. The swim bladder is an oxygen rich environment where blood is the only source of nutrition and there is no problem of osmolarity in this atypical biotope. The liver of the mammals is an oxygen poor environment where bile and liver tissue are the sources of nutrition, and osmolarity does not cause any effect on the parasite because the contents of the bile remain more or less constant; whereas in the rumen the oxygen tension remains very low,

food and osmolarity varies because both depend upon the nature of the diet of the host. In addition, one of the parasites lives in a poikilothermic and the other three parasites inhabit homeothermic animal. Such differences in the nature of habitats provide an opportunity to see how parasites have adapted to their respective habitats and how differences in habitats have resulted in the differences in their biochemical and physiological adaptations. It would be interesting to examine and study the physiological and biochemical similarities and differences between these parasites, which might exist as a result of host specificity and niche segregation. Biochemical diversity must receive as much attention as biochemical unity. Undue emphasis on the latter will lead to a distorted picture. Therefore comparative biochemistry should be concerned with the nature of variability from patterns common to many forms of life and in this manner should contribute to a better understanding of biochemical evolution and adaptation.

The facilities available to the author were very limited and work was done in a moderately equipped laboratory, therefore only some aspects of the respiratory metabolism have been studied. Still many problems of the respiratory metabolism have remained due to lack of facilities, for example, oxygen consumption under various tensions of oxygen, oxygen affinity with haemoglobin and

collagen, Pasteur, Crabtree, and Bohr effects, and terminal respiratory metabolism etc.

During the course of this study it was proposed to investigate the following various aspects of the respiratory metabolism of the four species of trematodes mentioned above: the normal oxygen consumption and the effect of temperature, pH, various substrates, chemicals, hormones, ions, osmotic stress, carbon monoxide, anaerobic incubation, Krebs cycle substrates, on the oxygen consumption of trematodes by using Warburg manometric technique.

It is hoped that whatever little work has been accomplished by the present investigator would stimulate further research work on this topic for a comparatively unstudied field of trematode biochemistry and physiology.

C H A P T E R - IV

MATERIALS AND METHODS

All experimental material was obtained from naturally occurring infections. Fresh trematodes were collected from the local abattoir and the fish market. Cotylophoron cotylophorum and Gastrothylax crumenifer were collected from the rumen and Gigantocotyle explanatum were collected from the liver of the Indian water buffalo, Bubalus bubalis L., soon after the animals were slaughtered. The worms were transferred to Tyrode solution (NaCl 136 mM; KCl 2.6 mM, $CaCl_2$ 1.8 mM; $NaHCO_3$ 1.1 mM; NaH_2PO_4 0.32 mM and $MgCl_2$ 0.9 mM), and were incubated for one to two hours in this medium which was adjusted at pH 7.5 in a water bath maintained at 37 $\pm$ 2°C, in order to shed their eggs and intestinal content. For Isoparorchis hypselobagri, infected swim bladders of the catfish, Wallago attu were brought to the laboratory and the worms were rinsed in the modified Ringer's saline (Forster and Taggart, 1950) containing NaCl 100 mM; KCl 2.5 mM; $CaCl_2$ 1.5 mM; $MgCl_2$ 1.0 mM; NaH_2PO_4 0.5 mM; $NaHCO_3$ 5 mM and were incubated for one to two hours in the same medium, which was adjusted at pH 7.5 in a water bath maintained at 25 $\pm$ 2°C to shed their eggs and hematin content of the intestinal caeca.

In all experiments, Tyrode was used for the mammalian trematodes and modified Ringer's for the fish trematode. According to Siddiqi, Islam and Nizami (1975) these salines are approximately isotonic to the trematodes under study. In the case of the mammalian trematodes, the oxygen consumption was determined at 37°C and pH 7.5 and in the case of the fish trematode at 30°C and pH 7.5 unless mentioned otherwise. Following the measurement of oxygen consumption, the worms were dried at 100°C for 24-36 hrs and the oxygen utilization has been expressed as $ul O_2$ consumed/mg dry wt/hr.

I. Normal Oxygen Consumption:

Aerobic endogenous respiratory rates were measured by Warburg manometry. Three to five flukes were placed in the main compartment of each 15 ml Warburg flask with 2.0 ml of saline. Filter paper fans soaked in 0.3 ml of 20% KOH were placed in the center well for the absorption of CO_2 gas. Respiration was measured by the direct Warburg method of Umbreit et al. (1964).

II. Effect of Temperature:

The metabolic temperature response in relation to oxygen consumption by the parasites was studied at 20°, 25°, 30°, 35°, 40° and 45°C. No worm was used more than once.

III. Effect of pH:

Study of the influence of pH was under taken on the oxygen consumption of fish and cattle trematodes. In order to obtain and maintain the intended hydrogen ion concentration of Tyrode and fish salines the various levels of pH from 5 to 8 were adjusted by 0.2M tris-maleate buffer and pH 9 to 11 were adjusted by 0.2M glycine-NaOH buffer. Three to five worms were removed from their habitats and kept for two hours in 5 ml saline in order to become adapted as far as possible to various levels of pH. After two hours, the oxygen consumption was determined as described above for a period of one hour. For each experiment a new batch of three to five worms was used. The results are expressed as ulO_2 consumed/mg dry wt/hr.

IV. Effect of Various Substrates:

In order to examine the effect of different substrates on oxygen consumption the following substances were used: glucose, fructose, mannose, lactose, galactose, maltose, ribose, -glycerophosphoric acid, glutamic acid, alanine and proline, in the concentration of 8 mM in salines of a total volume of 2 ml. Three to five adult worms were used in each flask. The observations were made at 10 minute intervals. A control without substrate was run in each experiment.

V. Effect of Various Chemicals:

The effect of the following different kinds of chemicals on oxygen consumption was studied: Diethyldithiocarbamate (0.005M); ethylurethane ($1x10^{-3}$M); salicylaldoxime (0.003M); sodium arsenite ($1x10^{-3}$M); phenylthiourea (0.01M); 2,4-dinitrophenol($1x10^{-4}$M); 2,4-dinitrocyclopentyl phenol ($1x10^{-4}$M); iodoacetate (0.001M); malonate (0.01M); potassium cyanide ($1x10^{-3}$M); p-chloromercuric benzoate (0.01M) and sodium fluoride (0.01M).

Freshly collected worms were starved for one hour in glucose free Tyrode and modified Ringer's prior to the experiment. Three to five adult flukes were used with 2 ml saline containing the inhibitor. A control in each experiment was run simultaneously without using the inhibitor.

VI. Effect of Various Hormones:

The following hormones were used in this experiment: Thyroxine (1 ug/ml), insulin (50 ug/ml), 5HT (serotonin) (10^{-4}M) histamine (10^{-4}M), adrenaline (10^{-4}M), noradrenaline (10^{-4}M), testosteron (10^{-4}M) and progesteron (10^{-4}M). Three to 5 flukes were used with 2 ml saline containing the hormone.

VII. Effect of Various Ions:

For the purpose of studying the effect of various ions on trematode oxygen consumption various salines were used for

mammalian trematodes and they were the same as devised by von Brand and Gibbs (1966) and contained the following composition along with 8 mM of glucose in each case.

1. Control: Normal Tyrode

	gm/lit.
NaCl	8.00
KCl	0.20
$NaHCO_3$	1.00
NaH_2PO_4	0.60
$MgCl_2\ 6H_2O$	0.20
$CaCl_2\ 2H_2O$	0.30

2. Sodium-free Tyrode

KCl	10.40
$KHCO_3$	1.20
KH_2PO_4	0.06
$MgCl_2\ 6H_2O$	0.20
$CaCl_2\ 2H_2O$	0.30

3. Low Sodium Tyrode (A)

KCl	10.40
$NaHCO_3$	1.00
KH_2PO_4	0.06
$MgCl_2\ 6H_2O$	0.20
$CaCl_2\ 2H_2O$	0.30

4. Low Sodium Tyrode (B)

NaCl	2.00
KCl	7.80
$NaHCO_3$	1.00
KH_2PO_4	0.06
$MgCl_2\ 6H_2O$	0.20
$CaCl_2\ 2H_2O$	0.30

5. Potassium-free Tyrode

NaCl	8.15
$NaHCO_3$	1.00
NaH_2PO_4	0.06
$MgCl_2\ 6H_2O$	0.20
$CaCl_2\ 2H_2O$	0.30

6. Magnesium-free Tyrode

NaCl	8.15
KCl	0.20
$NaHCO_3$	1.00
NaH_2PO_7	0.60
$CaCl_2\ 2H_2O$	0.30

7. Calcium-free Tyrode

NaCl	8.15
$NaHCO_3$	1.00
KCl	0.20
NaH_2PO_3	0.06
$MgCl_2\ 6H_2O$	0.20

8. Phosphate-free Tyrode

NaCl	8.00
KCl	0.20
$NaHCO_3$	1.00
$MgCl_2\ 6H_2O$	0.20
$CaCl_2\ 2H_2O$	0.30

9. High Calcium Tyrode

NaCl	7.30
KCl	0.20
$NaHCO_3$	1.00
NaH_2PO_4	0.06
$MgCl_2\ 6H_2O$	0.20
$CaCl_2\ 2H_2O$	3.00

10. High Magnesium Tyrode

NaCl	7.50
KCl	0.20
$NaHCO_3$	1.00
NaH_2PO_4	0.06
$MgCl_2\ 6H_2O$	2.00
$CaCl_2\ 2H_2O$	0.30

11. High Phosphate Tyrode

NaCl	7.50
KCl	0.20
$NaHCO_3$	1.00
NaH_2PO_4	0.60
Na_2HPO_4	1.60
$MgCl_2\ 6H_2O$	0.20
$CaCl_2\ 2H_2O$	0.30

Similarly fish saline was modified by either replacing or by decreasing or increasing the quantity of one cation with another.

Fish salines

1. Normal (control)	gm/lit.
NaCl	5.844
KCl	0.1865

$CaCl_2$	0.1660
$MgCl_2$	0.950
NaH_2PO_4	0.0590
NaH_2CO_3	0.4200

2. Sodium free saline

KCl	6.0305
$KHCO_3$	0.420
KH_2PO_4	0.590
$MgCl_2$	0.0950
$CaCl_2$	0.166

3. Low Sodium Saline

NaCl	1.000
KCl	5.0305
$NaHCO_3$	0.4200
KH_2PO_4	0.0590
$MgCl_2$	0.0950
$CaCl_2$	0.266

4. Low Sodium Saline

NaCl	2.000
KCl	4.0305
$NaHCO_3$	0.4200
KH_2PO_4	0.0590

$MgCl_2$	0.0950
$CaCl_2$	0.166

5. Potassium free saline

NaCl	6.0305
$CaCl_2$	0.1660
$MgCl_2$	0.0950
NaH_2PO_4	0.590
$NaHCO_3$	0.400

6. Magnesium free saline

NaCl	5.844
KCl	0.1865
$CaCl_2$	0.1660
NaH_2PO_4	0.0590
$NaHCO_3$	0.4200

7. Calcium free saline

NaCl	5.844
KCl	0.1865
$MgCl_2$	0.0950
NaH_2PO_4	0.0590
$NaHCO_3$	0.4200

8. Phosphate free saline

NaCl	5.844
KCl	0.1865
$NaHCO_3$	1.000
$MgCl_2$	0.20
$CaCl_2$	0.166

9. High Calcium Saline

NaCl	4.844
KCl	0.1865
$NaHCO_3$	0.4200
NaH_2PO_4	0.0590
$MgCl_2$	1.1660

10. High Magnesium Saline

NaCl	4.844
KCl	0.1865
$NaHCO_3$	0.04200
NaH_2PO_4	0.0590
$MgCl_2$	1.0950

11. High Phosphate Saline

NaCl	4.844
KCl	0.1865
$CaCl_2$	0.1660
$MgCl_2$	0.0950
NH_2PO_4	1.0590
$NaHCO_3$	0.4200

In preparing the high phosphate saline, care was taken to avoid precipitation of calcium phosphate by first dissolving it in water separately. It was added to the rest of the constituents only immediately before use. In each case, three to five flukes were placed in a Warburg flask with 2 ml of experimental saline containing 8 mM glucose. The control in each set was run simultaneously in normal Tyrode or fish saline containing 8 mM glucose.

VIII. Effect of Osmotic Stress:

The effect of osmotic stress on oxygen consumption has been studied in only three species: Gigantocotyle explanatum, Gastrothylax crumenifer and Isoparorchis hypselobagri.

In these experiments the oxygen consumption was studied in normal, diluted and concentrated salines containing 8 mM glucose. The saline concentrations were the same as used by Siddiqi et al. (1975). In the Warburg flask 2 ml of appropriate saline was taken along with 3 to 5 parasites of each species.

IX. Effect of Carbon Monoxide:

The effect of carbon monoxide has also been studied in three species of trematodes: Gigantocotyle explanatum, Gastrothylax crumenifer and Isoparorchis hypselobagri. Warburg manometers and flasks containing worms were first gassed with CO for 20, 40

and 60 minutes. In each flask 3 to 5 parasites were placed in 2 ml of saline containing glucose and the side arm stoppers of the flasks were kept open in order to replace air by CO. The CO was allowed to pass for about 5 minutes. The side arm stoppers of the flasks were closed first followed by stopcocks of the manometers. In CO gas phase, the parasites were incubated for 20, 40 and 60 minutes. After the incubation, both the openings of the manometer system were opened and then oxygen uptake measurements were made for one hour in the air phase. The results of oxygen uptake were compared with the oxygen uptake of control group of parasites which had not been incubated in the CO gas phase.

X. Effect of Oxygen Debt (Post Anaerobic Respiration):

Post anaerobic respiration was studied only in the case of Isoparorchis hypselobagri. Immediately after collection, 3 to 5 worms were placed in a Warburg flask in 2 ml saline containing 8 mM glucose. The flasks were attached to the manometers and nitrogen gas was introduced in the system in order to establish anaerobic conditions. The parasites were incubated in the anaerobic environment for 30, 60, 90 and 120 minutes. After anaerobic periods, the worms were returned to air by opening the manometer stopcock and were equilibrated for 10 minutes. Oxygen uptake was measured for six post anaerobic periods of 20 minutes

each. At the end of the post anaerobic respiration the worms were removed from the flask and dried at 100°C. A control was run in each experiment which was not introduced or exposed to the anaerobic environment.

XI. Effect of TCA Cycle Substrates:

In this experiment Isoparorchis hypselobagri was studied for the effect of TCA cycle substrates on oxygen consumption, and the following substrates were used:

Oxaloacetate, citrate, isocitrate, ∝-ketoglutarate, succinate, fumurate and malate. These substrates were made up in fish saline. Three to five flukes were used in each flask. A control without substrate was run in each experiment.

In summarising results for tabular presentation, data from each trial were arranged. Student 't' test (Simpson and Roe, 1939) was used to judge the significance of difference.

C H A P T E R - V

RESULTS AND DISCUSSION

I. Normal Oxygen Consumption:

The results of the oxygen consumption in the presence and absence of glucose have been summarized in Table I. It can be seen that all the four species of digenetic trematode under study have been found to consume oxygen, though at different rates. Isoparorchis hypselobagri from the swim bladder of the catfish consumed more oxygen than the mammalian trematodes. Among the mammalian trematodes, Gigantocotyle explanatum from the liver consumed more oxygen than the trematodes of the rumen; while among the rumen trematodes, Gastrothylax crumenifer consumed more oxygen than Cotylophoron cotylophorum.

The differences in the rates of oxygen uptake appear to be great if one looks at the QO_2 values, however, the same are reduced when one looks at the VO_2 values. It is probably the water content of the parasites which might be responsible for the higher rate of oxygen uptake by I. hypselobagri. The water content of the later is very high (92%) and it is not so

Table - I. Normal oxygen consumption in trematodes at pH 7.5

Species	Host/ location	Temp. ^{o}C	Dry wt : Wet wt	QO_2		VO_2
				Without glucose	With glucose	With glucose
Isoparorchis hypselobagri	Fish Swim bladder	30	1:11	3.926	6.214	0.501
Cotylophoron cotylophorum	Buffalo Rumen	35	1:2	0.125	0.171	0.052
Gastrothylax crumenifer	Buffalo Rumen	35	1:2	0.234	0.322	0.101
Gigantocotyle explanatum	Buffalo Liver	35	1:4	1.466	2.209	0.449

QO_2 = $ul O_2$/mg dry wt/hr ; VO_2 = $ul O_2$/mg wet wt/hr

very high in the cattle trematodes (70-80%) as reported by Siddiqi et al. (1975).

Previous studies on the oxygen consumption of trematodes indicate that the oxygen of the habitat of the parasite plays an important role. The parasite which lives in an oxygen rich environment consumes more oxygen than the parasite which inhabits low oxygen environments. The parasites of lung and blood, P. ohirai (Bruce et al., 1971), P. westermani (Shimomura, 1959 and Hamajima, 1973), P. miyazakii (Hamajima, 1972), S. mansoni (Bueding, 1950) and S. japonicum (Shimomura, 1959) consume more oxygen than the parasites of the liver, intestine or rumen, which have so far been studied (Table II). This contention appears to be true and is supported also by the results of the present investigation.

The difference in the normal oxygen consumption of trematodes under study is probably due to high and low oxygen tensions in the respective habitats. I. hypselobagri lives in an oxygen rich environment (Siddiqi and Nizami, 1974, 1975), while the other trematodes inhabit such environments where oxygen tension is poor. In the case of rumen trematodes, the different species of trematodes living side by side in the same habitat show a slight difference in their oxygen consumption. C. cotylophorum and G. crumenifer have QO_2 values of 0.125 and

0.234 respectively. However, Lazarus (1950) reported a QO_2 value of 0.03 for F. cervi, a parasite from rumen and Goil (1958) reported a QO_2 value of 0.319 for G. crumenifer. While QO_2 values of 3.5 and 3.7 have been reported for the parasites of intestine, F. revolutum (Taft and Fried, 1968) and Metagonimus yokagawai (Shimomura, 1959) respectively. Similarly, G. explanatum has a QO_2 value of 1.466. The previous studies on the oxygen consumption on trematodes of liver, C. sinensis (Bruce et al., 1971), D. dendriticum (Eckert and Lehner, 1971), F. hepatica (van Grenbergen, 1949 and Shimomura, 1959) and P. explanatum (Goil, 1959) are in agreement with the present study, except F. gigantica (Goil, 1961), where a QO_2 value of only 0.037 has been reported. Goil (1958) reported low oxygen consumption by F. gigantica as compared with P. explanatum and suggested that different rates of QO_2 in parasites of the same habitat may be due to the differences in their sizes. However, the size of the parasite is not probably so important as the water content and the pO_2 of the habitat. The latter as mentioned above is probably playing the most important role. If as a result of parasitic mode of life, the parasites have undergone morphological and anatomical changes (i.e., parasitic adaptations) they must have adapted themselves physiologically as well as biochemically. This is supported by the previous

studies (see Table II) which shows that the trematodes which live in environments with low pO_2 have low QO_2 values and those which inhabit environments with high pO_2 have higher QO_2 values. Over a very long period of parasitization of a particular niche, the parasites have become adapted to the latter and differences in the oxygen consumption can be a direct consequence of niche segregation. The available data on the response of organisms to varying oxygen tensions generally fall into two categories. In some animals the metabolic rate varies proportionately with the amount of oxygen present, such animals are called conformers. In others the respiratory rate remains relatively constant over a wide range of oxygen tensions until some low critical value (P_c) is reached, then the rate declines rapidly. These animals are said to be regulators (Vernberg, 1968). The metabolic rate of adult trematodes is generally dependent on the oxygen tension: S. mansoni (Bueding, 1949), F. hepatica (Harnisch, 1932), G. aduncа (Hunter and Vernberg, 1955; Vernberg, 1963). However, conflicting results have been reported by Harnisch (1932) and van Grenbergen (1949), while the former reported dependency of the metabolic rate on the oxygen tension, the latter worker found no dependency on the oxygen tension in the case of F. hepatica.

In spite of the fact that oxygen is necessary for the survival of F. hepatica, Stephanson (1947) found that anaerobic

conditions decreased the survival time of F. hepatica. Rohrbacher (1957) observed that although F. hepatica lives under nearly anaerobic conditions in the bile duct, it lost the colour of its haemoglobin when maintained anaerobically in vitro, whereas aerobic conditions do not produce such changes. This indicates that the oxygen is an important factor for the survival of these parasites which inhabit nearly anaerobic environments. Schistosoma mansoni can survive in anaerobiosis in vitro for 5 days but under aerobic conditions the worms lived 16 days (Ross and Bueding, 1950). I. hypselobagri is another example which lives in an oxygen rich environment, and the maximum survival under aerobic conditions was 49 days whereas under anaerobic conditions it survived only for 30 days (Nizami and Siddiqi, 1975). In vitro experiments on trematodes suggest that small amount of oxygen is essential for the growth and development for both groups of parasites.

The slight variations in oxygen uptake in trematodes from the same habitat is probably due to the differences in species, size and age of the worms, etc. Shimomura (1959) found that the proportions in which oxygen is consumed by the three different parasites in the same period of time are one for S. japonicum, about one-third for P. westermani and about

two-fifth for Metagonimus yokogawai. The low QO_2 value in Paragonimus species as compared with Schistosoma, although the former parasite inhabits lungs, is probably due to location in the cyst, even though lung is high in oxygen tension. Bruce et al. (1971) suggested that his results on Clonorchis sinensis vary from the results obtained by Nagamoto and Okaba (1957) on the same species and are due to the differences between the geographical strains of C. sinensis.

The significance of oxygen consumption in the metabolism of parasites is difficult to explain. It has been conjectured that oxygen may play a more important part in the production of intermediate compounds of metabolism than in the production of energy (Read, 1956), however, in some species, both these processes are likely to be occurring in parallel. Read (1956) suggested that "if oxygen is utilized by tissues, a concentration gradient from the peripheral to the central tissues will result; the tension in the central region may thus be zero. If by products of the central anaerobic metabolism could diffuse to the peripheral tissue, they could be oxidized there; conversely, peripherally oxidised products could be available, after diffusion in the centre." Usually oxygen is utilized in oxidation of carbon atoms to CO_2, in terminal oxidation processes. Both cytochromes and haemoglobin

have been reported among trematodes by a number of workers. Lutz and Siddiqi (1967) and Siddiqi and Haider (unpublished results) have presented convincing evidence that haemoglobin found in trematodes is a true porphyrin distinct from that of the host and is of endogenous origin. The actual function of these haemoglobins of trematodes is unclear. Manwell (1960) stated that although oxygen storage is the basic function of tissue haemoglobin, the significant aspect of this is that the pigment accepts oxygen molecule into chemical combination, which in turn facilitates the transport of oxygen to the tissue. He correlates this adaptation with the lack of active transport mechanism for oxygen. Manwell (1960) and Freeman (1963) have pointed out that the stored oxygen in the haemoglobin would not allow the organism to maintain aerobic metabolism for any length of time under anaerobic conditions. Lee and Smith (1965) reported that endoparasitic haemoglobin have such a high affinity for oxygen that they are completely saturated at oxygen tensions as low as 1 or 2 mm Hg partial pressures. Since these oxygen tensions are within the range normally encountered by the parasites. These authors also suggest that the chief function of the haemoglobin in parasites is to facilitate oxygen transfer rather than acting as an oxygen storage.

Table - II. Oxygen consumption of digenetic trematodes

Species	Location	VO_2	QO_2	References
P. ohirai	Lung		2.238	Bruce et al. (1971)
P. westermani	Lung		2.805* 2.0	Shimomura (1959) Hamajima (1972)
P. miyazaki	Lung	484		Hamajima (1972)
S. mansoni	Blood		6.0 8.7	Bueding (1950) Bueding (1950)
S. japonicum	Blood		10.33	Shimomura (1959)
H. medioplexus	Lung	0.452		Bair & Peters (1971)
I. hypselobagri	Swim bladder	0.501*'	6.21*	Present study
C. sinensis	Liver		2.28 6.5*	Bruce et al. (1971) Nagamoto & Okabe (1959)
D. dendriticum	Liver		1.1	Eckert & Lehner (1971)
F. hepatica	Liver	0.054- 0.112'		Harnisch (1932)
			1.9 1.485*	van Grembergen (1949) Shimomura (1959)
		0.261- 0.318'		Mansour (1959)
F. gigantica	Liver		0.037	Goil (1961)
P. explanatum	Liver		0.983	Goil (1958)
G. explanatum	Liver	0.441*'	2.209*	Present study
G. crumenifer	Rumen		0.319	Goil (1958)
P. cervi	Rumen	0.003'	0.03	Lazarus (1950)
C. cotylophorum	Rumen	0.052*'	0.171*	Present study
G. crumenifer	Rumen	0.101*'	0.322*	Present study

(Continued)

Table - II (Continued)

Species	Location	VO_2	QO_2	References
			3.5	Taft & Fried (1968)
E. revolutun	Intestine		3.795*	Shimomura (1959)
M. yokagawai	Intestine			Vernberg & Hunter (1963)
H. quissetensis	Intestine	200-600		Vernberg & Hunter (1959)
G. adunca	Intestine		0.032+	Vernberg & Hunter (1961)
S. beauforti	Intestine		0.040+	Vernberg & Hunter (1961)
P. malaclemys	Intestine		0.032+	Freeman (1962)
P. subtenuis	Kidney	0.168'	1.68	

VO_2 $ul O_2$ consumed/gm wet wt/hr

QO_2 $ul O_2$ consumed/mg dry wt/hr

\+ $ul O_2$ consumed/ug N/hr

' $ul O_2$ consumed/mg wet wt/hr

* in presence of glucose

II. Effect of Temperature:

The effect of temperature on the oxygen consumption of trematodes was studied at six different temperatures ranging from 20° to 45°C. The results have been summarized in Table III and Fig. 1. Increase in temperature results in gradual increase of QO_2 in I. hypselobagri, and beyond 40°C any increase in temperature is found to be quite detrimental to the parasite respiration. The optimum temperature appears to be 30°C at which temperature the QO_2 is twice as much as at 20°C. The metabolism of the fish parasite continues to be normal at lower temperature while the higher temperature (45°C) causes sudden decrease in oxygen uptake. In mammalian trematodes lower temperatures drastically retard the metabolic activity as shown by lower QO_2 values and increase in temperature causes ten to fifteen time increase in the oxygen uptake at an optimum temperature of 40°C. Any increase in temperature beyond 40°C is not so deterimental in the mammalian trematodes as it is in the case of fish trematode (Fig. 1). The optimum temperatures for oxygen consumption for fish and mammalian trematodes are 30°C and 40°C respectively. On either side of this temperature there is reduction in oxygen uptake in both sets of parasites. The metabolic response of the rumen and liver trematodes is identical. In the case of fish trematode, 45°C is more deterimental than it is to the mammalian

Table - III. Effect of temperature on oxygen consumption of trematodes in the presence of glucose

Species	Temperature					
	20°C	25°C	30°C	35°C	40°C	45°C
Isoparorchis hypselobagri	3.203± 0.021	4.733± 0.018	6.630± 0.031	6.080± 0.031	5.820± 0.017	1.430± 0.028
Cotylophoron cotylophorum	0.018± 0.002	0.039± 0.004	0.123± 0.004	0.167± 0.002	0.189± 0.003	0.141± 0.005
Gastrothylax crumenifer	0.023± 0.012	0.107± 0.003	0.202± 0.002	0.323± 0.003	0.375± 0.005	0.258± 0.004
Gigantocotyle explanatum	0.203± 0.015	0.443± 0.017	1.320± 0.027	2.147± 0.031	2.502± 0.071	2.278± 0.059

Values are in ulO_2 consumed/mg dry wt/hr

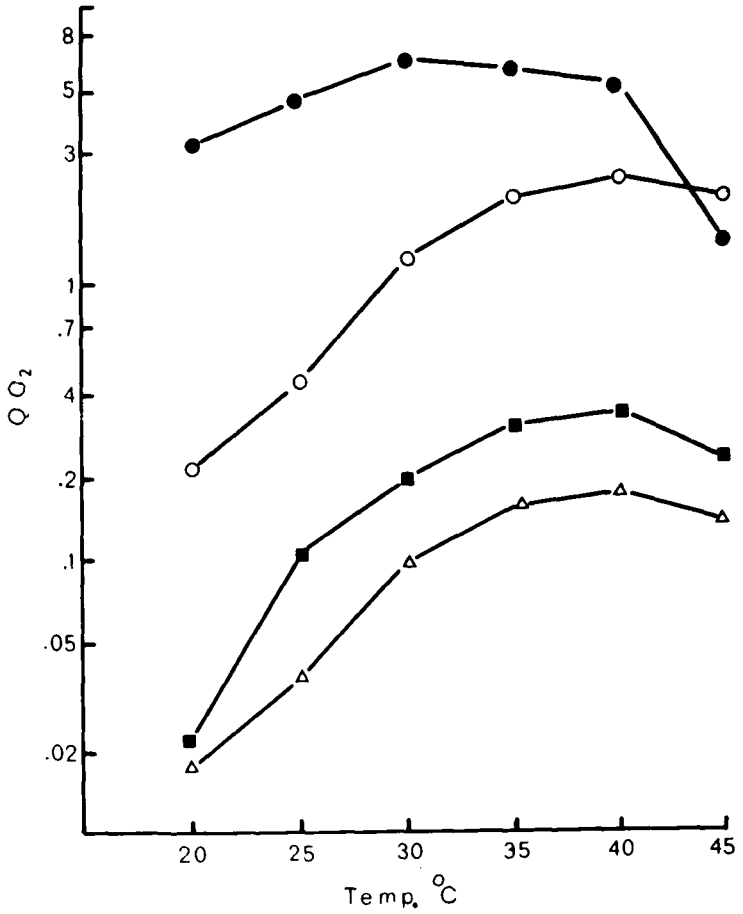

Fig. 1. Effect of temperature on the oxygen consumption of different species of trematodes.

● I. hypselobagi, ○ G. explanatum,

■ G. crumenifer, △ C. cotylophorum.

trematodes.

The optimum temperature for oxygen consumption is different in fish and mammalian trematodes as shown in Fig. 1, and is probably due to the fact that I. hypselobagri lives in a poikilothermic animal whereas C. cotylophorum, G. crumenifer and G. explanatum live in a homeothermic animal. The former is subject to many temperature variations. Our results indicate that the fish trematode remains more active and consumes more oxygen over a wider range of temperature, i.e., 20-35°C as compared to mammalian trematodes. Fry and Hochachka (1970) have suggested that two kinds of processes often occur during acclimatization of poikilothermic organisms to new temperature regimes: "(1) compensatory adjustment in the metabolic rate, which tends to free the organisms from the tyrany of their environment and (ii) biochemical reactions of many cellular components in order to maintain optimal activity at the new temperature."

The data concerning the effect of temperature on the oxygen uptake in trematodes is meagre and not many species from various hosts and habitats have been studied. The effect of temperature on the respiration is of considerable importance in parasites of poikilothermic animals, as Vernberg (1961 a,b)

and Vernberg and Hunter (1961) pointed out that once the temperature is raised a few degrees above the temperature to which the parasite is normally subjected in its natural habitat, the respiration rate is depressed. Vernberg and Hunter (1961) studied the effect of temperature in three trematodes, Gynaecotyle adunca from the bird showed increasing respiration rate up to 41°C; the turtle parasite, Pleurogonimus malaclemys showed increased oxygen uptake up to 36°C, and the fish parasite Saccocoelium beauforti, showed an increased respiration rate only up to 30°C. The results of the present investigation are in agreement with these results. It is also interesting to note that the respiration of the fish inhabiting immature Schistocephalus solidus increases up to 40°C since the adult is a bird parasite (Davies and Walkey, 1966). It seems probable that this tolerance to high temperature is genetically determined (Vernberg and Hunter, 1961).

The results of the present study support the view of Vernberg and Hunter (1961) that the metabolic-temperature response of parasites closely parallel the body temperature of their respective hosts and is evident not only at higher temperatures but also at low temperatures. Such type of studies would be interesting to examine similarities and

differences between the parasites of poikilothermic and homeothermic animals, which might exist as a consequence of niche specialization. Rao and Bullock (1954) suggested that the temperature of the habitat influences the respiration of the free living animals. Vernberg and Hunter (1961) believe that this has also proved to be true for endoparasites.

The data concerning the influence of temperature on the respiration of trematode will prove to be fruitful in further research on the comparative biochemical and physiological aspects of trematodes, especially in respect to in vitro culture of these animals.

III. Effect of pH:

The results of the effect of pH on oxygen consumption of trematodes are given in Table IV and Fig. 2. It is apparent that the oxygen consumption of the fish, rumen and liver trematodes remain more or less unaltered from pH 7 to 9, 6 to 9 and 8 to 10 respectively. Any increase or decrease in these ranges of pH undoubtedly has adverse effect on the oxygen consumption of these trematodes. This clearly indicates that the wide range of pH 6 to 9 is more or less optimum pH at which rumen trematodes consume oxygen at a steady rate. This is probably because the rumen trematodes are subjected to frequent changes of their surrounding medium and thus seem to be better adapted to a wider range of pH. On the other hand G. explanatum being a parasite of the bile duct, is adapted to a much narrower limit of pH levels since bile is a more constant medium than the contents of the cattle rumen.

In all four species under study, the acidic pH is more deterimental to the oxygen uptake than the alkaline pH. The oxygen uptake by the trematodes in the acidic range of pH is low as compared with the respiration in the alkaline range of pH. The degree of stimulation or inhibition of oxygen uptake in acidic or alkaline pH in trematodes of swimbladder,

Table - IV. Effect of pH on oxygen consumption of trematodes in the presence of glucose

Species	°C	pH							
		4.0	5.0	6.0	7.0	8.0	9.0	10.0	11.0
Isoparorchis hypselobagri	30	2.493± 0.211	4.015± 0.190	5.821± 0.372	6.393± 0.327	6.617± 0.361	6.501± 0.210	5.106± 0.350	4.372± 0.101
Cotylophoron cotylophorum	35	0.061± 0.036	0.092± 0.021	0.167± 0.013	0.173± 0.010	0.178± 0.005	0.171± 0.021	0.164± 0.060	0.117± 0.013
Gastrothylax crumenifer	35	0.133± 0.013	0.239± 0.026	0.331± 0.031	0.341± 0.013	0.353± 0.026	0.351± 0.041	0.304± 0.034	0.271± 0.042
Gigantocotyle explanatum	35	1.010± 0.103	1.390± 0.113	1.630± 0.125	1.960± 0.108	2.370± 0.230	2.560± 0.090	2.420± 0.136	2.150± 0.188

Values are ulO_2 consumed/mg dry wt/hr

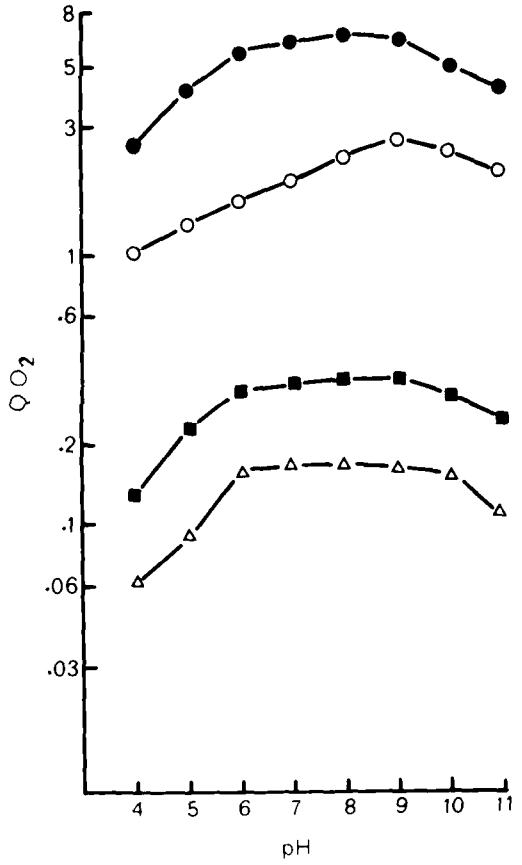

Fig. 2. Effect of pH on the oxygen consumption of different species of trematodes.

● I. hypselobarri, ○ G. explanatum,

■ G. crumenifer, △ C. cotylophorum.

rumen and liver is different, it is probably related to the differences in habitat, because the response of rumen trematodes at various levels of pH is identical, however, the fish and liver trematodes remain more active in the alkaline range of pH whereas the rumen trematodes show increased oxygen uptake from pH 6-9.

However, von Brand (1952) suggested that if the respiration remains unchanged over a wide pH range, one can not necessarily assume that their protoplasm is insensitive to such changes; it is perhaps protected by the external membrane.

While working with Schistosoma mansoni, Bueding (1950) found that the respiration remains more or less unchanged at a pH range of 7.0 to 8.7. Below pH 7.0 the oxygen uptake decreases and also any increase in the level of pH above 8.7 resulted in a less sharp reduction in the rate of respiration. von Brand (1973) pointed out that the optimum pH range for the respiration of parasites remains approximately unaltered and varies from species to species. It is evident from the present study that the rumen trematodes show a greater range in which their rate of respiration remains unchanged. This is due to the fact that the pH of the rumen depends upon the diet of the host. Similarly the liver trematodes show maximum oxygen uptake in an alkaline range, since the pH of the

bile is alkaline. I. hypselobarri behaves similar to S. mansoni and its respiration remains unaffected between pH 7 to 9 (Bueding, 1950). The swim bladder is a simple but not much explored biological niche where blood, whose composition is known, is the only source of nutrition for the trematode parasite. The only difference between S. mansoni and I. hypselobarri is that the former lives intravascularly and the latter extravascularly. The results of the present study and of previous studies on helminth parasites clearly indicate that the respiration of parasites depend upon the pH of the surrounding medium or microenvironment in which these parasites live, and supports the fact that the nature of the habitat has influenced the biochemical and physiological characteristics of the parasite living in that habitat.

IV. Effect of Various Substrates:

Once it was established that trematodes consume oxygen, it was decided to examine the role of various substrates on their oxygen uptake. The results of these studies are summarized in Tables V - IX and Figs. 3-7.

The effects of various hexoses (glucose, fructose, galactose and mannose) on the oxygen consumption are more or less similar in the four species of trematodes under study. Among the various sugars used, glucose is most effective in stimulating the oxygen uptake (33% to 53%) while galactose is least effective (10% to 17%). The respiration of I. hypselobagri was stimulated more than the other three species under investigation in the presence of these hexoses. Among mammalian trematodes the respiration of the liver trematode, G. explanatum was stimulated more than of the rumen trematodes.

The effect of disaccharides (lactose and maltose) on the respiration of trematodes have also been studied, and lactose stimulates respiration in all the four species of trematodes, but maltose had little or no stimulatory effect on any of the species under study. It had a depressing effect in Cotylophoron cotylophorum and Gastrothylax explanatum which

was, however, found to be statistically insignificant.

The only pentose sugar used was ribose. It has but little stimulatory effect on the oxygen consumption which varies from 8-12%.

Another interesting point which comes to light from the present study is that, in spite of the fact that the four species are subjected to similar concentrations of substrates, the percent change in oxygen consumption is different in every trematode. It also indicates that the rate of utilization is different which may be due to differences in metabolism.

Preferential utilization of hexoses on the part of Gigantocotyle explanatum may be due to the fact that it lives in the liver where glucose is in abundance. G. explanatum has become adapted to this feature and can absorb more glucose than other sugars. Maltose was not found to stimulate oxygen uptake in any species of trematodes under study. This finding is not in agreement with that of Stephanson (1947), who hold the opinion that maltose increases survival time of F. hepatica when added to the medium, which means that the liver fluke makes use of maltose. However, the result of the present study suggests that in these trematodes, amylomaltase (trans, 1-4-glu-

Table - V. Summary of results of the effect of various substrates on the oxygen consumption of trematodes

Substrates	Percent change of initial rate			
	I. hypselobagri	*C. cotylophorum*	*G. crumenifer*	*G. explanatum*
1. Glucose	+53.84	+33.44	+39.31	+40.99
2. Fructose	+44.67	+31.20	+36.32	+37.17
3. Mannose	+41.87	+30.24	+24.35	+33.28
4. Galactose	+17.93	+10.40	+13.67	+17.12
5. Maltose	+ 3.41	- 2.40	+11.53	- 5.66
6. Lactose	+28.37	+20.64	+29.91	+23.53
7. Ribose	+ 9.67	+ 8.48	+12.39	+ 9.82
8. Glutamic acid	+30.43	+14.40	+23.07	+31.03
9. Alanine	+24.80	+17.84	+20.51	+28.37
10. Proline	+18.95	+13.76	+11.53	+30.28
11. Glycerol	+43.14	+40.80	+46.58	+55.59
12. α-GPA	+28.37	+25.60	+34.18	+26.87

Table - VI. Effect of various substrates on oxygen consumption of I. hypselobagri

Substrate	n	$Q O_2$		% Change	p Value	Significance*
		Mean	SE			
Control	8	3.926	±0.062			
Glucose	8	6.040	±0.045	+53.84	< 0.001	+++
Fructose	10	5.680	±0.058	+44.67	< 0.001	+++
Mannose	9	5.570	±0.038	+41.87	< 0.001	+++
Galactose	6	4.630	±0.005	+17.93	< 0.001	+++
Maltose	10	4.060	±0.020	+ 3.41	< 0.25	-
Lactose	6	5.045	±0.289	+28.37	<0.02	++
Ribose	8	4.300	±0.064	+ 9.67	=0.05	+
Glutamic acid	6	5.121	±0.168	+30.43	<0.005	++
Alanine	7	4.900	±0.138	+24.80	< 0.005	++
Proline	6	4.670	±0.106	+18.95	< 0.01	++
Glycerol	7	5.620	±0.018	+43.14	<0.001	+++
α-GPA	5	5.040	±0.190	+28.37	<0.01	++

* - Insignificant, + Significant, ++ Highly significant,
+++ Very highly significant

Table - VII. Effect of various substrates on oxygen consumption of C. cotylophorum

Substrate	n	$Q O_2$		% Change	p Value	Significance*
		Mean	SE			
Control	10	0.125	±0.001			
Glucose	9	0.168	±0.002	+33.40	< 0.001	+++
Fructose	7	0.164	±0.003	+31.20	< 0.001	+++
Mannose	7	0.1628	±0.002	+30.24	< 0.001	+++
Galactose	9	0.138	±0.003	+10.40	< 0.02	++
Maltose	5	0.122	±0.002	- 2.40	< 0.5	-
Lactose	8	0.150	±0.004	+20.64	< 0.005	++
Ribose	6	0.135	±0.002	+ 8.48	< 0.05	+
Glutamic acid	6	0.143	±0.019	+14.40	< 0.01	++
Alanine	8	0.147	±0.003	+17.84	< 0.005	++
Proline	8	0.142	±0.004	+13.76	< 0.02	++
Glycerol	7	0.176	±0.003	+40.80	< 0.001	+++
α-GPA	6	0.157	±0.003	+25.60	< 0.001	+++

* - Insignificant, + Significant, ++ Highly significant,
+++ Very highly significant

Table - VIII. Effect of various substrates on oxygen consumption of G. crumenifer

Substrate	n	QO_2		% Change	p Value	Significance*
		Mean	SE			
Control	8	0.234	±0.003			
Glucose	7	0.326	±0.001	+39.31	< 0.001	+++
Fructose	6	0.319	±0.003	+36.32	< 0.001	+++
Mannose	6	0.291	±0.008	+24.35	< 0.005	++
Galactose	6	0.266	±0.008	+13.67	=0.05	+
Maltose	6	0.261	±0.009	+11.53	< 0.2	-
Lactose	5	0.304	±0.019	+29.91	< 0.05	+
Ribose	6	0.263	±0.010	+12.39	< 0.02	++
Glutamic acid	5	0.288	±0.012	+23.07	< 0.025	++
Alanine	6	0.282	±0.009	+20.51	< 0.02	++
Proline	7	0.261	±0.005	+11.53	< 0.05	+
Glycerol	6	0.343	±0.004	+46.58	< 0.001	+++
α-GPA	7	0.314	±0.016	+34.18	< 0.01	++

* - Insignificant, + Significant, ++ Highly significant

+++ Very highly significant

Table - IX. Effect of various substrates on oxygen consumption of G. explanatum

Substrate	n	$Q O_2$		% Change	p Value	Significance*
		Mean	SE			
Control	8	1.466	±0.031			
Glucose	9	2.067	±0.039	+40.99	< 0.001	+++
Fructose	8	2.011	±0.106	+37.17	< 0.02	++
Mannose	7	1.954	±0.132	+33.28	=0.05	+
Galactose	8	1.717	±0.053	+17.12	=0.05	+
Maltose	6	1.383	±0.011	- 5.66	<0.2	-
Lactose	6	1.811	±0.083	+23.53	<0.05	+
Ribose	6	1.610	±0.014	+ 9.82	<0.05	+
Glutamic Acid	7	1.921	±0.080	+31.03	<0.02	++
Alanine	7	1.882	±0.113	+28.37	<0.02	++
Proline	8	1.910	±0.082	+30.28	<0.02	++
Glycerol	8	2.281	±0.163	+55.59	<0.02	++
α-GPA	8	1.860	±0.076	+26.87	<0.02	++

* - Insignificant, + Significant, ++ Highly significant,
+++ Very highly significant

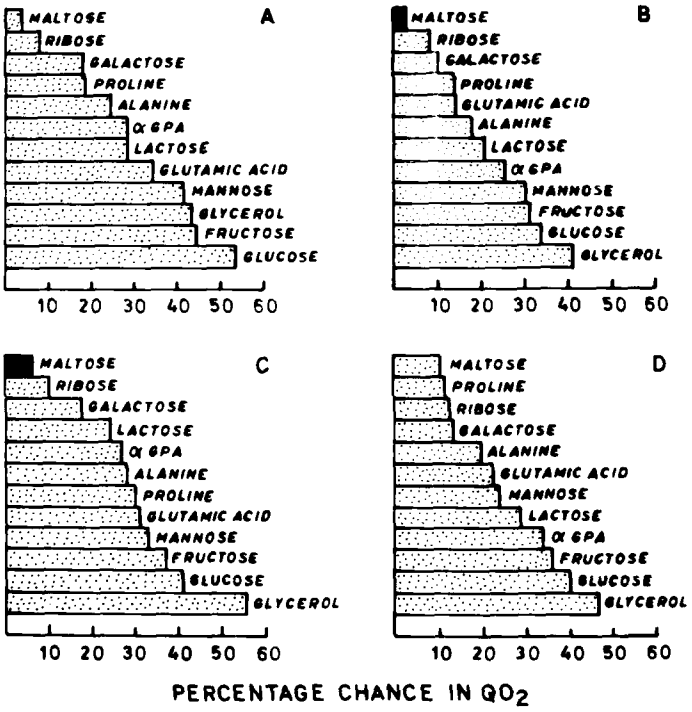

Fig. 3. Effect of various substrates on oxygen consumption of trematodes.

A. I. bypseloba ri, B. C. cotylophorum,

C. G. explanatum, D. G. crumenifer.

Percent increase Percent decrease

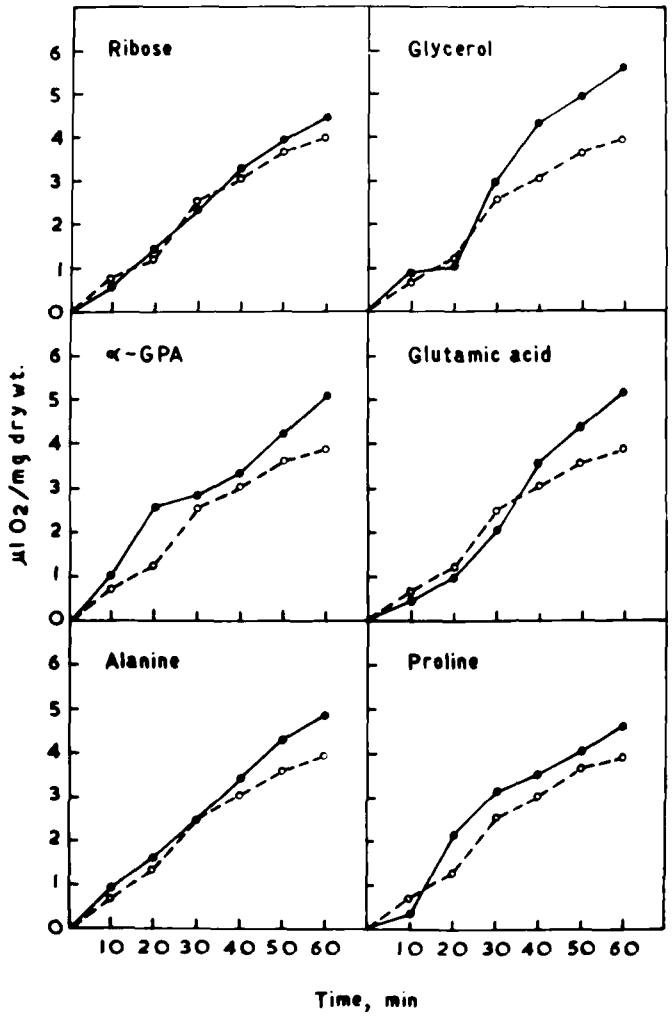

Fig. 4b. Influence of various substrates on oxygen consumption as a function of time in I. hypselobarri.

--- CONTROL
——— WITH SUBSTRATE

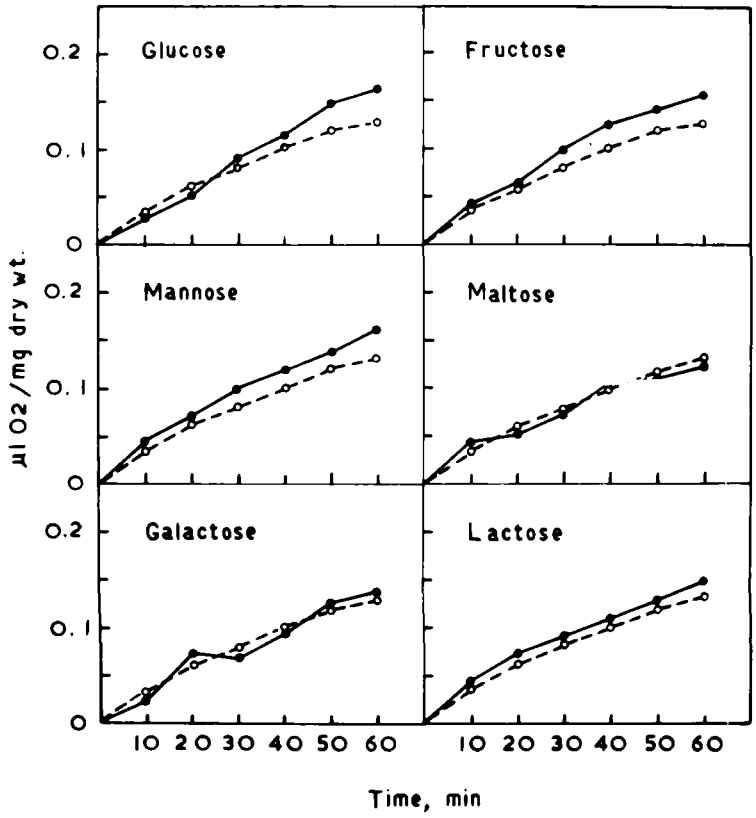

Fig. 5a. Influence of various substrates on oxygen consumption as a function of time in C. cotylophorum.

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——— WITH SUBSTRATE

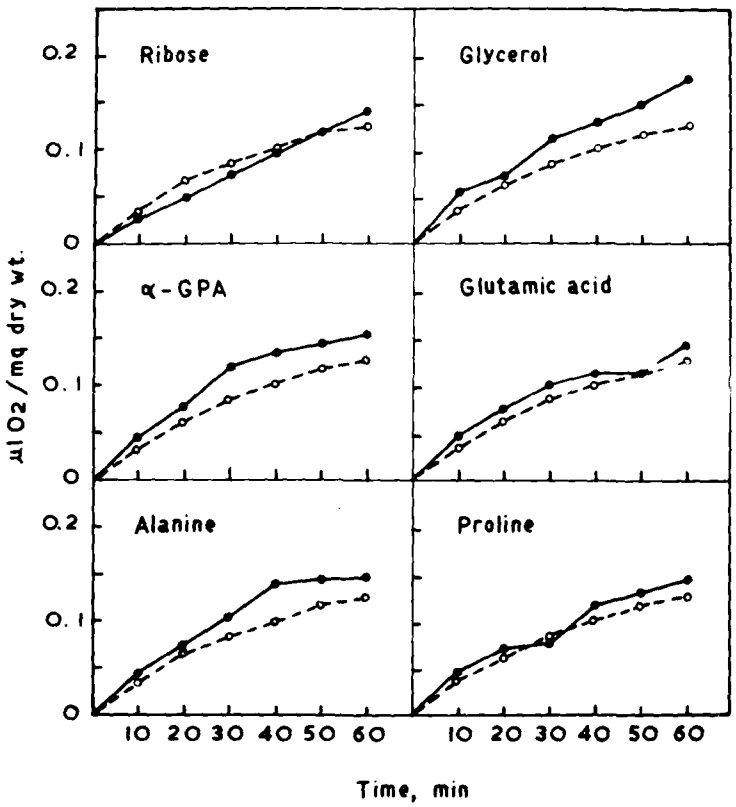

Fig. 5b. Influence of various substrates on oxygen consumption as a function of time in C. cotylonhorum.

--- CONTROL
—— WITH SUBSTRATE

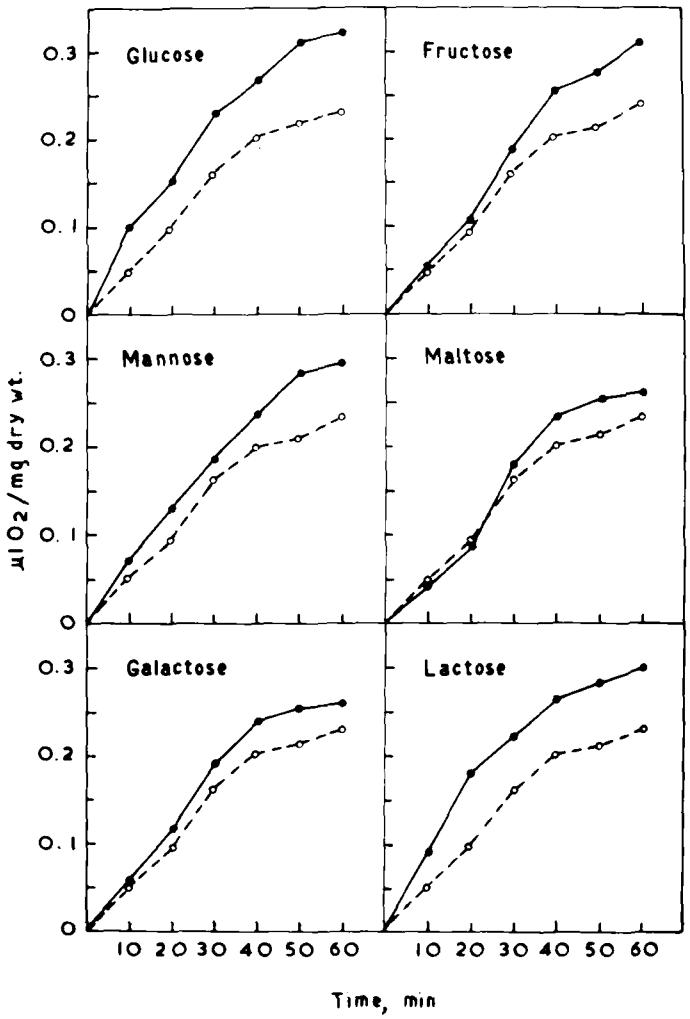

Fig. 6a. Influence of various substrates on oxygen consumption as a function of time in G. crumenifer.

- - - CONTROL
——— WITH SUBSTRATE

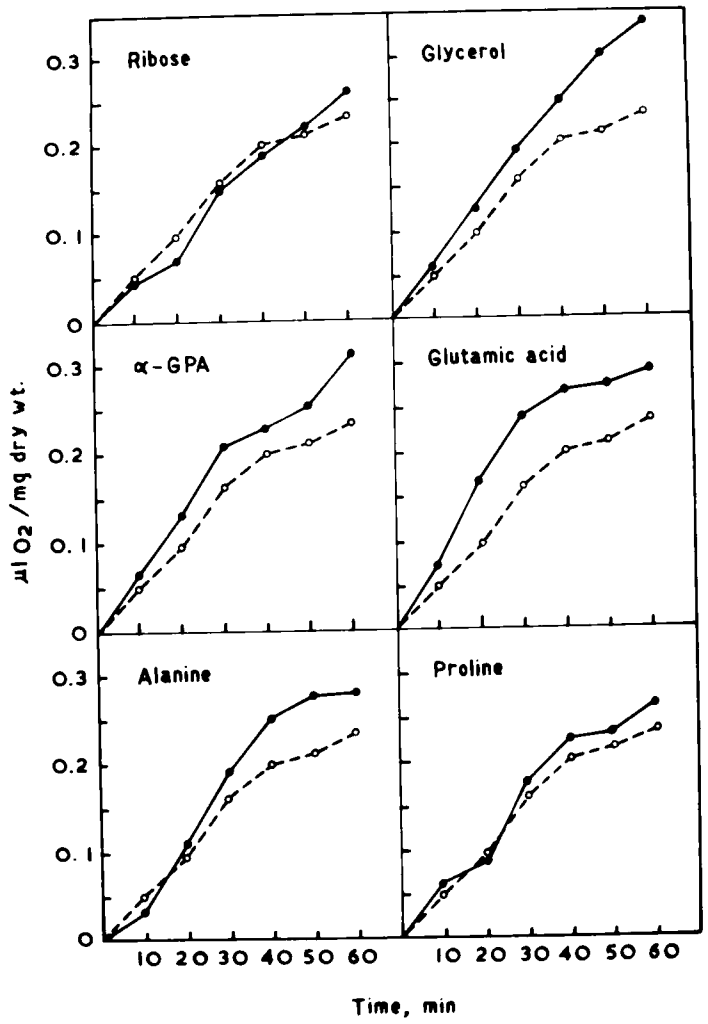

Fig. 6b. Influence of various substrates on oxygen consumption as a function of time in G. crumenifer.

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——— WITH SUBSTRATE

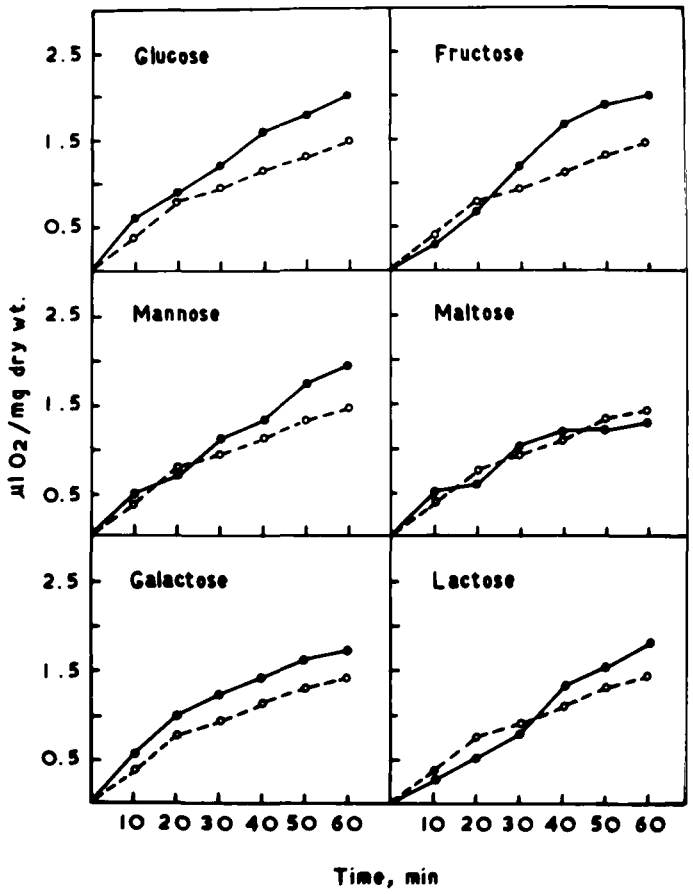


Fig. 7a. Influence of various substrates on oxygen consumption as a function of time in G. exolanetum.

--- CONTROL

—— WITH SUBSTRATE

coselyase) enzyme responsible for digestion of maltose is absent. The results of the effect of various substrates on the respiration of these worms suggests that different enzymes are responsible for the phosphorylation and utilization of these substrates, as Buedin; et al. (1954) have shown that in S. mansoni the phosphorylation of glucosamine and of glucose are catalyzed by two different enzymes.

von Brand (1973) pointed out that "various criteria have been used to identify the amino acids absorbed by parasites: appearence of radioactivity in the tissues after incubation in media containing labelled amino acids, demonstration that a given environmental amino acid decreases in concentration during incubation period, that it leads to increased ammonia production, or to a significant increase in oxygen consumption over the endogenous rate". The effect of the three amino acids, glutamic acid, alanine, and proline was also studied on the respiration of the trematodes. These amino acids cause a marked increase in the respiration of the trematodes. Comparatively, the oxygen uptake in G. explanatum was greatly stimulated by amino acids than in the fish and the rumen trematodes. Among the rumen trematodes, the respiration of G. crumenifer was stimulated more than in C. cotylophorum in the presence of these amino acids. The oxygen uptake of I. hyposelobagri was

stimulated more by glutamic acid than by alanine or proline.

The respiration of both fish and mammalian trematodes was greatly stimulated in the presence of glycerol than in the presence of carbohydrates and amino acids, and the maximum stimulation (55%) was found in G. explanatum. Among the rumen trematodes again, marked stimulation took place in the respiration of G. crumenifer than of C. cotylophorum. α - glycerophosphate had more stimulatory effect (34%) in G. crumenifer than other species of trematodes under investigation.

It has been shown by Islam (1974) that glucose, fructose, sorbose and mannose uptake in C. cotylophorum, G. crumenifer and G. explanatum is highest in the first hour of incubation. It was therefore decided to examine the pattern of oxygen consumption in the presence of various substrates in the first hour. The data on the influence of various substrates on oxygen consumption as a function of time is given in Figs. 4 to 7. The results clearly indicate that the relationship of oxygen uptake with the passage of time is more or less linear in the presence of various substrates. Generally the increase of oxygen uptake in the presence of substrates was slow in the first half hour than in the second half hour.

In all the four species under investigation, the effect of various substrates except maltose were found to be stimulatory up to the end of the experiment, though the extent of stimulation of oxygen uptake in every trematode is different. This is probably due to species differences or due to differences in nutritional requirements of these trematodes. If oxygen consumption is used as a parameter of hexose utilization than glucose and fructose were found to be more stimulatory and easily utilized than amino acids in all the species under study.

In vitro culture and respiration studies show that the few trematode species that have been studied are able to utilize simple carbohydrates more efficiently. Stephenson (1947), Rohrbacher (1957) and Mansour (1958, 1959) have demonstrated that the addition of glucose to saline media extends the in vitro survival of adult flukes. Bueding (1952) suggested that S. mansoni survives equally well in vitro, in media containing glucose, or mannose, but ribose, arabinose, galactose, sucrose, maltose or lactose are not beneficial. It seems probable that at least some of the trematodes may have the ability to utilize some of the carbohydrates. It was found in the present investigation that all species make use of carbohydrates, but they exhibit species difference and are adapted to utilizing one sugar

better than others. However, C. cotylophorum and G. crumenifer appear to be quite similar, as far as the utilization of sugars is concerned when compared with I. hypselobagri and G. explanatum. These differences in the results help in understanding the species differences in trematodes either from the same or from different habitats living in the same or different hosts. In the presence of various substrates except maltose higher QO_2 values are obtained when compared with the control, a finding that suggests that these substrates are readily taken up and utilized as energy source in the metabolism of trematodes.

There have been few studies on the carbohydrate metabolism of digenetic trematodes. Bryant and Williams (1962) demonstrated that the miracidium as well as the adult stage of F. hepatica utilizes glucose although differences were observed. Similarly Friedl (1961 a,b) reported that the survival of Fascioloides magna was prolonged by the addition of amino acids but simple sugars were without effect, although it has been demonstrated that adult helminths utilize glucogenic amino acids for glycogen synthesis (von Brand, 1960), Vernberg and Hunter (1963) found that glutamic acid, proline, glucose and mannose when added to the medium cause a marked increase in QO_2 while ribose has no effect on the respiration of adult Himasthala

quissentensis. van Grembergen (1949) found that glucose and alanine had no effect on oxygen uptake while ∝-glycerophosphoric acid and glutamic acid had strong stimulation of oxygen uptake by F. hepatica. Eckert and Lehner (1971) found that glucose, galactose, and fructose had no stimulatory effect while glycerol increased the oxygen consumption of Dicrocoelium dendriticum. However, glucose and glycerol when added to the sugar free Tyrode in various concentrations was absorbed by D. dendriticum during aerobic and anaerobic conditions. Bruce et al. (1971) reported that glucose, glucosamine, maltose, fructose, lactose, mannitol, mannose and galactose cause a significant increase in oxygen consumption of Paragonimus ohirai. Bueding (1950) found a slight increase in QO_2 value of paired S. mansoni in the presence of glucose. Read and Yogore (1955) reported a QO_2 value of 0.74 -0.86 for P. westermani with glucose, while Shimomura (1959) reported a value of 2.8. In those worms in which oxygen consumption decreases by the addition of carbohydrates it is probably due to the "Crabtree" effect (Bruce et al. 1971). This effect is produced by the increased competition of the anaerobic glycolytic process for inorganic phosphate and possibly pyridine nucleotides, leaving less for oxidative phosphorylation reactions (West and Todd, 1964; Fruton and Simmond, 1963). While

working on the nutritional requirements of larval form of Fascioloides magna, Friedl (1961a) suggested that the amino acids must be involved either with the nutrition or the protection of larval trematodes. Increased oxygen consumption in the presence of various substrates suggests that these are readily taken up and utilized as an energy source. By looking at the results of the present investigation, one can safely conclude that the trematodes have food preferences, and if many sugars are available, they are capable of making use of them to some extent according to their preference. Such information will be helpful in devising better culture media for trematodes.

V. Effect of Chemicals:

The effect of several inhibitors and stimulators on oxygen consumption of trematodes was examined and the results are given in the Tables X to XIV and Figs. 8 and 9. Table X shows the percent inhibition or stimulation in the four species of trematodes, whereas Tables XI-XIV summarize the data of individual species along with statistical analysis.

It was noticed that the chemicals used in this study were found to act as "inhibitor" except 2,4-dinitrophenol and 2,4-dinitrocylopentyl phenol though some are better and more effective than others, as can be seen by the extent of decrease in oxygen uptake. Among all the chemicals tested in the present study in the respiration of trematodes, potassium cyanide was found to be more inhibitory in all the four species of trematodes while diethyldithiocarbamate was found to be least effective in trematodes under investigation. Ethylurethane, salicylaldoxime, sodium arsenite, 2,4-dinitrophenol, 2,4-dinitrocyclopentyl phenol, iodoacetate malonate, potassium cyanide, p-chloromercuric benzoate were found to be more effective in influencing the respiration in fish trematode than in the mammalian trematodes. It is interesting to note that a particular chemical used in the case of all the four species in equal concentration was found to be similar in

action i.e., either stimulatory or inhibitory in all the four species under study but the extent of inhibition or stimulation in the four species under study is more or less of the same order with few exceptions. 2,4-dinitrocyclopentyl phenol is most stimulatory in the respiration of fish trematode as compared with mammalian trematodes. Similarly phenylthiourea is more inhibitory while sodium arsenite has least inhibitory effect in the oxygen consumption of G. explanatum as compared with other trematodes under study. Fig. 8 shows that KCN is the most effective and diethyldithiocarbamate is the least effective in all the four species of trematodes.

The overall effect of diethyldithiocarbamate on the oxygen consumption is inhibitory. It causes 17-25% inhibition in oxygen uptake by trematodes. Maximum inhibition was noticed in G. explanatum (24.98%) and I. hypselobagri (22.9%) while in the rumen trematodes it has less effect. Vernberg and Hunter (1960) found that diethyldithiocarbamate depressed the oxygen uptake by 60% in G. adunca. Jodrey and Wilbur (1955) reported 57% inhibition in oxygen uptake in oyster mantle in the presence of this compound and suggested an involvement of a copper respiratory catalyst. The effectiveness of copper inhibitors suggest a possible role in tyrosine

Table - X. Summary of results of the effect of various chemicals on oxygen consumption of trematodes

Additive	Percent change of initial rate			
	I. hymselobagri	C. cotylophorum	G. crumenifer	G. explanatum
1. Diethyldithio-carbamate	-22.99	-17.29	-18.80	-24.98
2. Salicylaldoxime	-58.49	-40.45	-48.62	-53.39
3. Phenylthiourea	-38.99	-43.53	-41.55	-54.70
4. Ethylurethane	-70.90	-63.53	-70.18	-67.26
5. Sodium arsenite	-61.17	-52.48	-56.33	-48.21
6. 2,4-dinitrophenol	+76.28	+70.67	+74.77	+61.48
7. 2,4-dinitrocyclopentyl-phenol	+78.09	+66.16	+62.84	+65.96
8. Iodoacetate	-64.25	-64.58	-59.63	-56.35
9. Malonate	-61.30	-53.70	-56.37	-58.62
10. Potassium cyanide	-83.40	-69.69	-72.34	-79.70
11. p-chloromercuric-benzoate	-77.17	-62.18	-66.97	-71.99
12. Sodium fluoride	-36.98	-27.52	-27.52	-30.97

Table - XI. Effect of various chemicals on oxygen consumption of *I. hypselobarri*

Additive	n	QO_2 Mean	QO_2 SE	% Change	p Value	Significance*
Control	8	4.031	±0.105			
Diethyldithiocarbamate	6	3.104	±0.151	-22.99	< 0.05	+
Salicylaldoxime	8	1.673	±0.468	-58.49	< 0.005	++
Phenylthiourea	4	2.459	±0.217	-38.99	< 0.01	++
Ethylurethane	7	1.169	±0.587	-70.90	< 0.02	++
Sodium arsenite	5	1.565	±0.749	-61.17	< 0.05	+
2,4-dinitrophenol	6	7.106	±0.548	+76.28	< 0.005	++
2,4-dinitrocyclopentyl phenol	6	7.179	±0.544	+78.09	< 0.005	++
Iodoacetate	8	1.441	±0.675	-64.25	< 0.05	+
Malonate	9	1.560	±0.171	-61.30	< 0.001	+++
Potassium cyanide	9	0.669	±0.125	-83.40	< 0.001	+++
p-chloromercuricbenzoate	7	0.920	±0.467	-77.17	< 0.02	++
Sodium fluoride	5	2.540	±0.200	-36.98	< 0.05	+

* - Insignificant, + Significant, ++ Highly significant, +++ Very highly significant

Table - XII. Effect of various chemicals on oxygen consumption of C. cotylophorum

Additive	n	QO_2 Mean	QO_2 SE	% Change	p Value	Significance*
Control	10	0.133	±0.011			
Diethyldithiocarbamate	7	0.110	±0.004	-17.29	=0.05	+
Salicylaldoxime	7	0.079	±0.003	-40.45	< 0.01	++
Phenylthiourea	6	0.075	±0.394	-43.53	< 0.05	+
Ethylurethane	8	0.048	±0.005	-63.53	< 0.005	++
Sodium arsenite	5	0.063	±0.010	-52.48	=0.05	+
2,4-dinitrophenol	9	0.227	±0.020	+70.67	=0.05	+
2,4-dinitrocyclopentyl phenol	10	0.221	±0.016	+66.16	< 0.02	++
Iodoacetate	9	0.047	±0.015	-64.58	< 0.05	+
Malonate	9	0.061	±0.005	-53.70	< 0.01	++
Potassium cyanide	9	0.040	±0.003	-69.69	< 0.001	+++
p-chloromercuricbenzoate	7	0.050	±0.016	-62.18	=0.05	+
Sodium fluoride	9	0.096	±0.001	-27.74	=0.05	+

* - Insignificant, + Significant, ++ Highly significant, +++ Very highly significant

Table - XIII. Effect of various chemicals on oxygen consumption of G. crumenifer

Additive	n	QO_2		% Change	p Value	Significance*
		Mean	SE			
Control	10	0.218	±0.006			
Diethyldithiocarbamate	8	0.177	±0.339	-18.80	< 0.005	++
Salicylaldoxime	5	0.112	±0.038	-48.62	< 0.05	+
Phenylthiourea	7	0.127	±0.025	-41.55	=0.05	+
Ethylurethane	7	0.065	±0.033	-70.18	<0.02	++
Sodium arsenite	5	0.095	±0.003	-56.33	<0.001	+++
2,4-dinitrophenol	9	0.381	±0.041	+74.77	=0.025	++
2,4-dinitrocyclopentyl phenol	7	0.355	±0.030	+62.84	<0.02	++
Iodoacetate	7	0.088	±0.036	-59.63	<0.05	+
Malonate	9	0.095	±0.025	-56.37	<0.02	++
Potassium cyanide	9	0.060	±0.009	-72.34	<0.001	+++
p-chloromercuricbenzoate	7	0.072	±0.041	-66.97	<0.05	+
Sodium fluoride	5	0.158	±0.016	-27.52	<0.05	+

* - Insignificant, + Significant, ++ Highly significant, +++ Very highly significant

Table - XIV. Effect of various chemicals on oxygen consumption of G. explanatum

Additive	n	QO_2		% Change	p Value	Significance*
		Mean	SE			
Control	9	1.989	±0.127			
Diethyldithiocarbamate	7	1.492	±0.031	-24.98	=0.05	+
Salicylaldoxime	9	0.927	±0.221	-53.39	<0.05	+
Phenylthiourea	5	0.901	±0.193	-54.70	<0.02	++
Ethylurethane	8	0.651	±0.051	-67.26	<0.001	+++
Sodium arsenite	6	1.030	±0.101	-48.21	<0.05	+
2,4-dinitrophenol	6	3.210	±0.063	+61.38	<0.001	+++
2,4-dinitrocyclopentyl phenol	7	3.301	±0.260	+65.96	<0.025	++
Iodoacetate	7	0.868	±0.157	-56.35	<0.02	++
Malonate	9	0.823	±0.223	-58.62	<0.05	+
Potassium cyanide	9	0.403	±0.125	-79.70	=0.05	+
p-chloromercuricbenzoate	5	0.557	±0.401	-71.99	<0.01	++
Sodium fluoride	4	1.373	±0.028	-30.97	<0.05	+

* - Insignificant, + Significant, ++ Highly significant, +++ Very highly significant

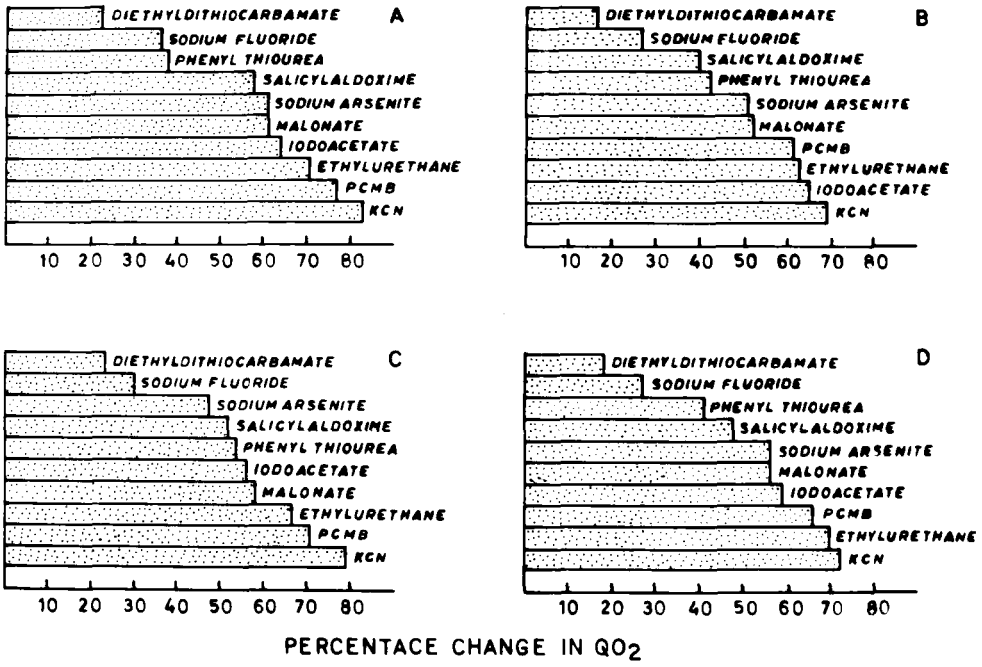


Fig. 8. Effect of various chemicals on oxygen consumption of trematodes.

A. I. hypselobagri. B. C. cotylophorum.

C. G. explanatum. D. G. crumenifer.

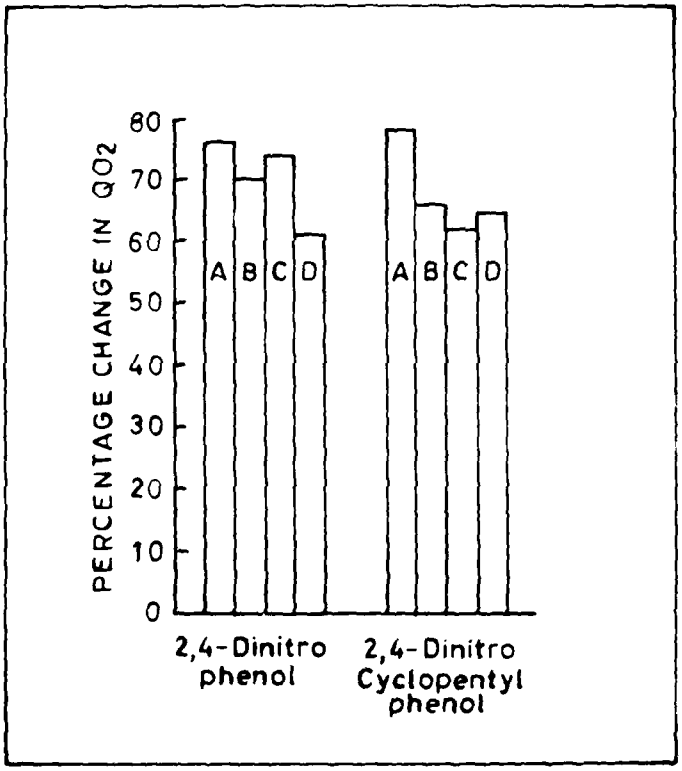


Fig. 9. Effect of 2,4-dinitrophenol and 2,4-dinitrocyclopentyl phenol on the respiration of trematodes.

A. I. hymselobarri, B. C. cotylophorum,

C. G. crumenifer, D. G. explanatum.

metabolism. Mansour (1958) found that diethyldithiocarbamate inhibits phenol oxidase activity in F. hepatica.

Salicylaldoxime causes maximum inhibition of oxygen uptake by I. hypselobagri (58.4%), followed by the liver trematode (53.9%). It was found to be slightly less inhibitory (43-48%) in the case of rumen trematodes. Salicylaldoxime is also a copper inhibitor. Vernberg and Hunter (1960) reported 58% reduction in oxygen uptake in adult G. adunca, while respiration in D. dendriticum was also inhibited by salicylaldoxime (Eckert and Lehner, 1971).

Phenylthiourea has least effect in I. hypselobagri, while the respiration of liver trematode in the presence of phenylthiourea was inhibited by 54.7%. In the rumen trematodes the inhibition of oxygen uptake was 41-43%. Phenylthiourea is also a copper inhibitor. Vernberg and Hunter (1960) found that the respiration of G. adunca was depressed 56% in the presence of phenylthiourea.

The inhibition of oxygen uptake in trematodes by ethylurethane ranges between 65 to 70%. The maximum inhibition was noticed in I. hypselobagri and G. crumenifer (70%), while in C. cotylophorum the inhibition is 63%. Ethylurethane ($NH_2.CO.OC_2H_5$) is cytotoxic and weakly hypnotic in action and

usually depresses the general metabolism. van Grenbergen (1949) reported a strong inhibiting effect of ethylurethane in the respiration of F. hepatica.

In the presence of sodium arsenite the oxygen consumption of the trematodes was found to be inhibited by 52-60%. The maximum inhibition was noticed in I. hypselobagri (61.17%). In S. mansoni also the rate of respiration is depressed by arsenite (Bueding, 1950). Sodium arsenite usually inhibits oxidative decarboxylation of pyruvate and α-oxo glutarate by combining with the dithiols of lipoic acid.

The oxygen uptake of four species of trematodes under study is stimulated in the presence of 2,4-dinitrophenol. The maximum stimulation was noticed in I. hypselobagri, followed by the rumen and the liver trematodes. Quite pronounced stimulatory effect was also noticed in F. hepatica (van Grenbergen, 1949). Eckert and Lehner (1971) reported that the rate of respiration of Dicrocoelium dendriticum was increased by 2,4-dinitrophenol, probably due to uncoupling of oxidative phosphorylation. 2,4-dinitrophenol, which exerts a general calorigenic action upon metabolism, has been found to increase lactic acid production and consequently increase the rate of carbohydrate oxidation.

The oxygen consumption of the trematodes was also stimulated in the presence of 2,4-dinitrocyclopentylphenol. The maximum stimulation was noticed in I. hypselobagri, while in the mammalian trematodes stimulation of oxygen uptake was recorded ranging from 62-66%. van Grenbergen (1949) also reported stimulatory effect of 2,4-dinitrocyclopentylphenol on the respiration of F. hepatica.

In the presence of iodoacetate, the oxygen consumption by I. hypselobagri and C. cotylophorum was inhibited by 64% while in G. crumenifer and G. explanatum it was only 59.6% and 56.3% respectively. Iodoacetate is a useful inhibitor reacting fairly specifically with SH groups present in the enzyme.

$$R - SH + CH_2.I.COOH \longrightarrow R-S-CH_2- COOH + HI$$

This inhibitor will block enzymes with a free sulphydryl group in the active site and in muscle tissue. The enzymes most sensitive to iodoacetate are glycraldehyde phosphate dehydrogenase which is a key enzyme in the breakdown of glycogen to lactate and triosephosphate dehydrogenase.

Malonate is the most effective inhibitor of the TCA cycle and in trematodes it causes a noticeable reduction in

the oxygen uptake. In the present study it was found to cause 53-61% inhibition in oxygen uptake of trematodes under study, and the extent of inhibition is more or less similar in all trematodes under study.

Malonate competitively inhibits succinic dehydrogenase. van Grenbergen (1949) reported that malonate inhibits the respiration of F. hepatica. Similarly Vernberg and Hunter (1960, 1963) found that respiration of G. adunca and Himasthla quissetensis was inhibited by malonate.

The maximum inhibition in oxygen uptake of trematodes was noticed in the presence of potassium cyanide, however, the later is more inhibitory in I. hypselobagri (83.4%), compared with the mammalian trematodes where it causes only 66-71% inhibition of the oxygen uptake.

Potassium cyanide has a property to combine with cytochrome oxidase, inactivate it, and thereby quickly stops a large proportion of cellular oxidation. Our results on KCN inhibition are in agreement with the results obtained by other workers with different species of trematodes: F. hepatica (van Grenbergen, 1949), S. mansoni (Buedin;, 1950; Ross and Buedin;, 1950; Buedin; and Charms, 1951; Buedin; et al. 1953) and D. dendriticum (Eckert and Lehner, 1971). However, Lazarus'

(1950) report is in disagreement with the present as well as the previous findings. Lazarus (1950) found that cyanide stimulated rather than depressed the respiration rate of Paramphistomum cervi. The validity of this report is doubtful and must be reinvestigated. As far as cyanide inhibition is concerned it can be explained by KCN interference of a respiratory enzyme or coenzyme concerned with the transfer of electrons, or the actual blockage of the cytochrome system. On the other hand, the over all oxygen consumption can be cut down to various degrees depending upon the availability of alternate pathways, if a compound inhibits a certain metabolic sequence above the stage where hydrogen is activated. However, Bueding (1949) has pointed out that stimulation or inhibition is not specific enough to really prove the absence or presence of a functional cytochrome system. It should be emphasized that cyanide inhibition gives no clear indication as to the nature of the respiratory system present.

In the presence of p-chloromercuric benzoate, the oxygen uptake was inhibited by 62-77% in trematodes under study. The maximum inhibition was recorded again in I. hypseloba ri, while mammalian trematode the respiration was inhibited 62-71%. The respiration rate of S. mansoni was also depressed by p-chloromercuric benzoate (Bueding, 1950). Benzoic acid usually

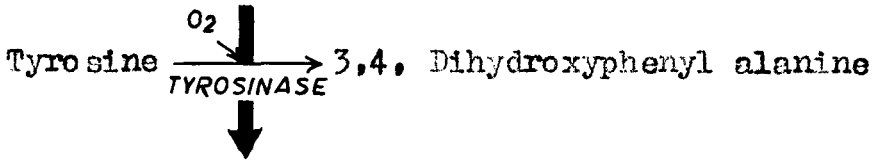
inhibits L-glycerol-3-phosphate oxidoreductase and glucokinase reaction.

Sodium fluoride inhibits the oxygen uptake to the extent of 27-37% in trematodes under study. Bueding (1950) found that fluoride produces a generalized depression in respiration of schistosomes. The results obtained in the present study confirms these findings of Bueding (1950). When fluoride is added to an actively glycolyzing system, the enolase is inactivated, probably because of the formation of a magnesium fluorophosphate complex. It seems that such type of study will be helpful in understanding the host parasite relationship as far as the chemotherapeutic studies are concerned. Islam (1974) found that glucose utilization by Gigantocotyle explanatum and Gastrothylax crumenifer was greatly inhibited in the presence of p-chloromercuric benzoic acid, KCN, sodium fluoride, and iodoacetate, although the present work does not deal with glucose utilization of the worms, the important relationship between respiration and glucose utilization is obvious.

The degree of stimulation or inhibition of oxygen uptake caused by various chemicals, is probably dependent upon the differential permeability of the tegument of trematodes, which appears to be a consequence of parasitism in different habitats.

As far as the mechanism of action of these chemicals is concerned in stimulating or inhibiting oxygen uptake, one can make several speculations. One of the possibilities may be that the inhibitors destroy the cellular structure of the tegument by causing denaturation of the proteins of the plasma membrane. Secondly, these compounds act as enzyme inhibitors in metabolic pathways and depress the oxygen utilization.

Study of the effect of inhibitors on the respiration of trematodes provides an opportunity to predict about the metabolic pathways in these organisms. However, the role of copper inhibitors in depressing the respiration rate is unclear. All the three copper inhibitors, which were used in the present study have been demonstrated to be inhibitors of tyrosinase (Lardy, 1949).



This clearly indicates that probably tyrosinase metabolism exists in trematodes. As far as diethyldithiocarbamate is concerned it inhibits phenol oxidase activity.

Inhibition of oxygen uptake by sodium arsenite indicates that the trematodes possess a mechanism which helps in the oxidative decarboxylation of pyruvate or α-oxaglutarate and also a

probability of the existence of lipoic acid in trematodes.

The iodoacetate inhibition indicates that parasites may have glyceraldehyde phosphate dehydrogenase, (which helps in the break down of glycogen to lactate) and triosephosphate dehydrogenase. Existence of succinic dehydrogenase, L-glycerol-3-phosphate oxidoreductase, glucokinase and enolase, can be predicted by the inhibition in oxygen uptake by malonate, p-chloromercuric benzoate and sodium fluoride. The mechanism of cyanide inhibition is well known, generally cyanide inhibits cytochrome a_3:

$$\text{Cyt } C_1 - \text{Cyt C} - \text{Cyt a} - \text{Cyt } a_3 \xrightarrow{O_2} H_2O$$
$$\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad \Big| \text{KCN}$$

This indicates that trematodes might have a cytochrome system. These studies of inhibition by various chemicals provide an indirect evidence that various enzymes and substrates, which are inhibited by these chemicals might exist in trematodes. However, it must be stressed that conclusion based on the use of inhibitors must be interprated with caution. Further resolution of this problem awaits the elucidation of metabolic pathways, and quantitative studies on these aspects need to be carried out.

VI. Effect of Hormones:

The present experiment was designed to examine the role of the hormones of the host whether they have any influence on the oxygen uptake of the parasites. Three species of trematodes, I. hypselobagri from the fish, G. crumenifer from the rumen and G. explanatum from the liver of the water buffalo were chosen for this study. The results are given in Tables XV to XVIII and Figs. 10-12. Table XV shows the percent inhibition or stimulation in the three species of trematodes, whereas Tables XVI-XVIII summarize the data on individual species along with statistical analysis.

It has been observed that the hormones used in the study were found to act as inhibitor or stimulator with the exception of noradrenaline, testosteron and progesteron which appeared to have statistically insignificant effect. It is interesting to note that a particular hormone used in the case of all the three species in equal concentration was found to be similar in action, i.e., either stimulatory or inhibitory in action in all the three species, but the extent of inhibition or stimulation is more or less of the same order with the exception of insulin in the case of G. explanatum, which shows increased oxygen uptake in the presence of insulin. This stimulatory effect of insulin on the liver parasite is due to the fact, that being a

parasite of liver it may be more sensitive to the action of insulin than the other parasites.

Thyroxine at a concentration of 1 ug/ml caused increase in oxygen uptake by trematodes under study. The maximum stimulation was noticed in G. explanatum (68%) and least stimulation was observed in I. hypselobagri (51.7%). The effect of thyroxine on the respiration of trematode is more or less similar. The data on the influence of thyroxine on oxygen consumption as a function of time is given in Fig. 10-12. These results clearly indicate that oxygen consumption of the trematodes increases with the passage of time.

Recently Cornford (1974) reported similar results in two other trematodes, Schistosomatium douthitti and Haematoloechus sp., and suggested that the effect is proportionate to the concentration of thyroxine. Previous studies with free living invertebrates showed that ciliate protozoans Tetrahymena gellui consumed more oxygen when treated with thyroxine, although Jenkins (1961) found no effect of thyroxine on Dugesia dorotocephala, but later on Cornford (1974) reported significant increase in oxygen uptake by Dugesia and Tetrahymena. While Hutton et al. (1972) found that thyroxine has no stimulatory effect on the metabolism of F. hepatica. Cornford (1974)

Table - XV. Summary of results of the effect of various hormones on oxygen consumption in trematodes

Additive	Percent change of initial rate		
	I. hypselobagri	G. crumenifer	G. explanatum
1. Thyroxine	+51.72	+63.24	+68.15
2. Insulin	+ 5.21	+11.06	+19.43
3. 5-HT (Serotonin)	+62.38	+71.14	+66.09
4. Histamine	-77.03	-90.90	-80.31
5. Adrenaline	-27.80	-35.57	-38.54
6. Noradrenaline	-21.49	-36.75	-29.43
7. Testosteron	- 2.61	- 8.69	- 5.21
8. Progesteron	- 3.97	-11.85	- 7.33

Table - XVI. Effect of various hormones on oxygen consumption in I. hypselobagri

Additive	n	$Q0_2$		% Change	p Value	Significance*
		Mean	SE			
Control	8	4.280	±0.105			
Thyroxine	6	6.494	±0.046	+51.72	< 0.001	+++
Insulin	9	4.503	±0.022	+ 5.21	< 0.25	-
5-HT (Serotonin)	7	6.950	±0.681	+62.38	< 0.05	+
Histamine	6	0.983	±0.703	-77.03	< 0.01	++
Adrenaline	6	3.090	±0.229	-27.80	< 0.05	+
Noradrenaline	5	3.360	±0.271	-21.49	=0.1	-
Testosteron	7	4.168	±0.231	- 2.61	< 0.9	-
Progesteron	8	4.110	±0.178	- 3.97	< 0.7	-

* - Insignificant, + Significant, ++ Highly significant, +++ Very highly significant

Table - XVII. Effect of various hormones on oxygen consumption in G. crumenifer

Additive	n	QO_2		% Change	p Value	Significance*
		Mean	SE			
Control	10	0.253	±0.021			
Thyroxine	9	0.413	±0.068	+63.24	< 0.001	+++
Insulin	6	0.281	±0.002	+11.06	< 0.05	+
5-HT (Serotonin)	7	0.433	±0.025	+71.14	< 0.02	++
Histamine	9	0.023	±0.009	-90.90	<0.001	+++
Adrenaline	6	0.163	±0.003	-35.57	< 0.05	+
Noradrenaline	7	0.160	±0.027	-36.75	=0.2	-
Testosteron	7	0.231	±0.004	- 8.69	<0.7	-
Progesteron	5	0.233	±0.009	-11.85	<0.7	-

*- Insignificant, + Significant, ++ Highly significant, +++ Very highly significant

Table - XVIII. Effect of various hormones on oxygen consumption in G. explanatum

Additive	n	QO_2		% Change	p Value	Significance*
		Mean	SE			
Control	8	1.746	±0.117			
Thyroxine	7	2.936	±0.085	+68.15	< 0.001	+++
Insulin	8	2.087	±0.001	+19.43	=0.05	+
5-HT (Serotonin)	9	2.900	±0.097	+66.09	< 0.005	++
Histamine	10	0.239	±0.239	-86.31	=0.01	++
Adrenaline	5	1.073	±0.076	-38.54	< 0.05	+
Noradrenaline	7	1.232	±0.222	-29.438	< 0.3	-
Testosteron	4	1.655	±0.045	- 5.31	<0.8	-
Progesteron	5	1.618	±0.137	- 7.33	<0.8	-

* - Insignificant, + Significant, ++ Highly significant, +++ Very highly significant

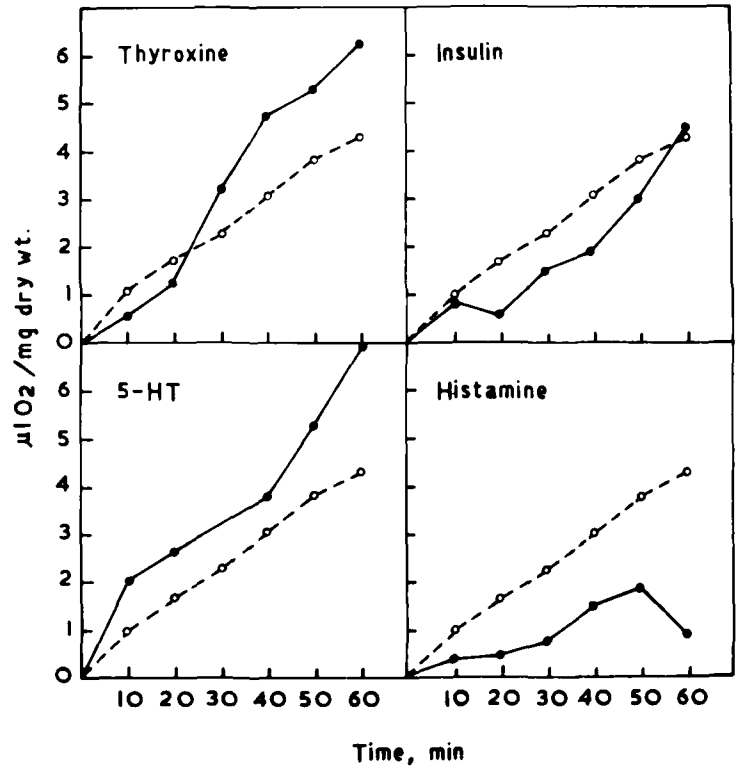


Fig. 10a. Influence of various hormones on oxygen consumption as a function of time in I. hyacelobagri.

--- CONTROL
—— WITH HORMONE

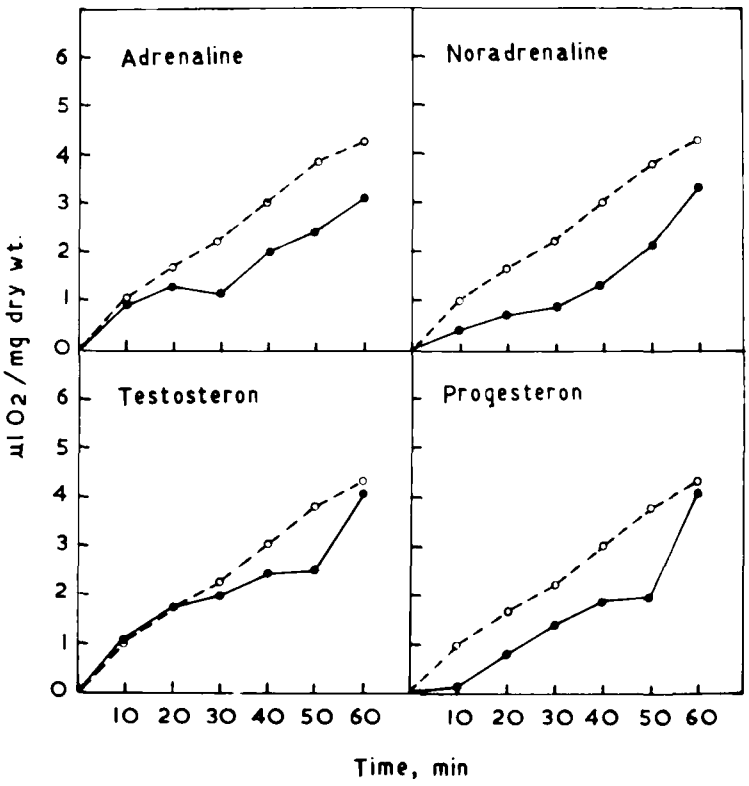


Fig. 10b. Influence of various hormones on oxygen consumption as a function of time in I. hypselobarri.

--- CONTROL
—— WITH HORMONE

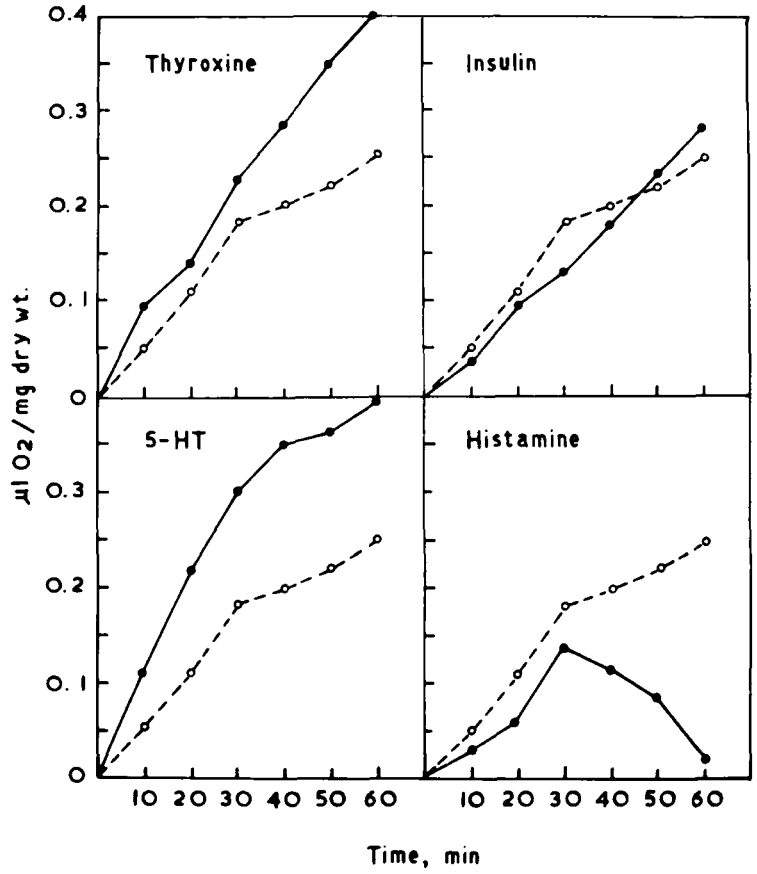


Fig. 11a. Influence of various hormones on oxygen consumption as a function of time in G. crumenifer.

- - - CONTROL
—— WITH HORMONE

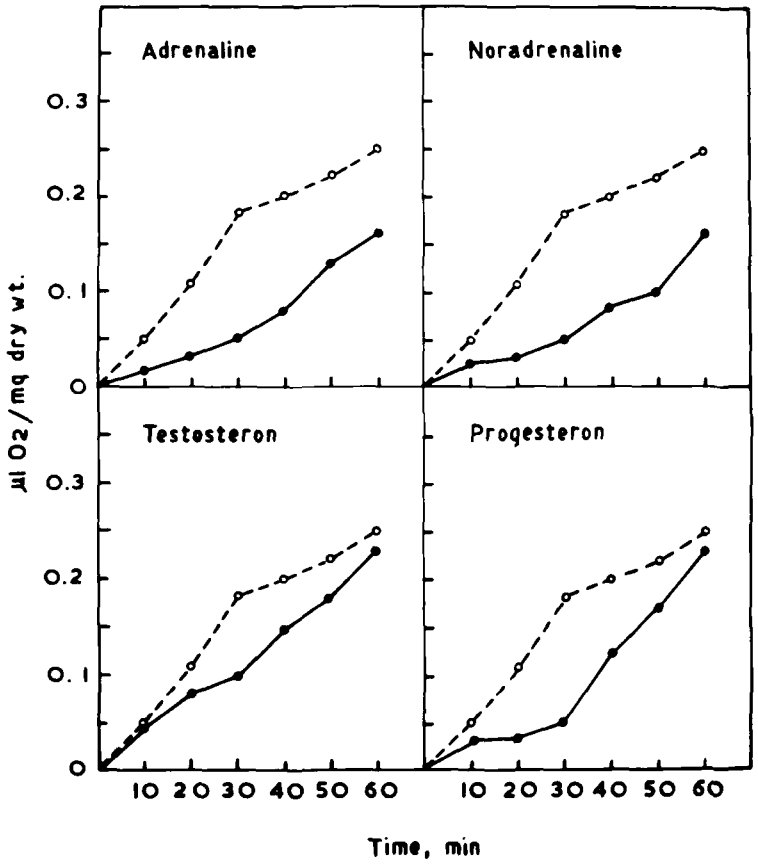


Fig. 11b. Influence of various hormones on oxygen consumption as a function of time in G. crumenifer.

- - - CONTROL
—— WITH HORMONE

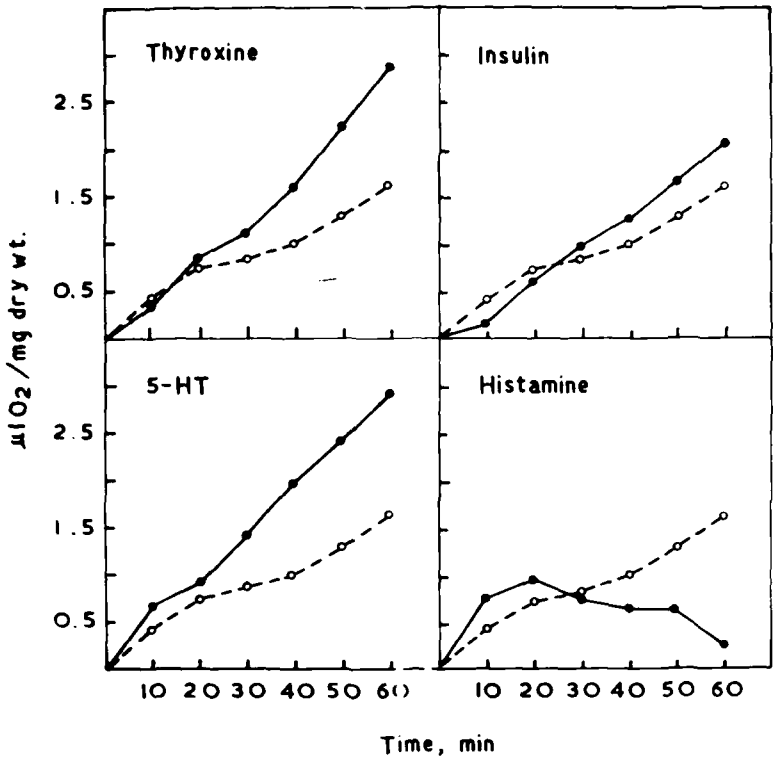


Fig. 12a. Influence of various hormones on oxygen consumption as a function of time in G. explanatum.

---- CONTROL
——— WITH HORMONE

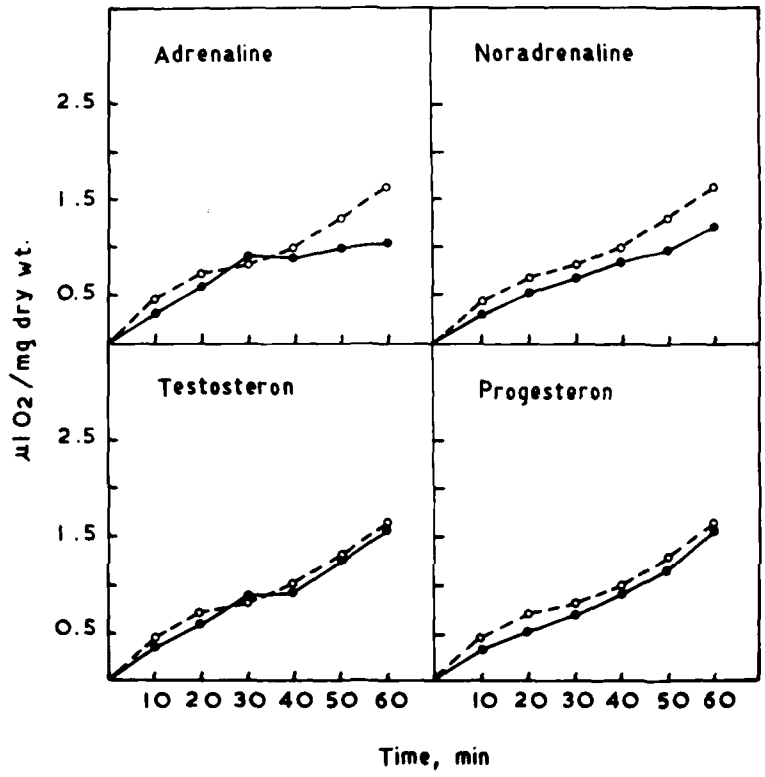


Fig. 12b. Influence of various hormones on oxygen consumption as a function of time in C. explanatum.

--- CONTROL

——— WITH HORMONE

found increased tetrazolium and cytochrome oxidase activity in Schistosomatium douthitti, and suggested that worms from hyperthyroid mice were significantly longer than comparable controls. Abdul Wahab et al. (1971) also found larger S. mansoni from hyperthyroid hosts. However, Pantelouris (1965) and Hutton et al. (1972) have failed to observe positive effects of this hormones in the liver fluke.

The results of the present study as well as on Schistosomatium douthitti established the fact that thyroxine certainly stimulates the respiration of trematodes as it did in all other invertebrates examined up till now. Increased oxygen consumption by trematodes in the presence of thyroxine indicates that this hormone may be playing an important role in their metabolism.

It is interesting to note that insulin has stimulatory effect on the respiration of mammalian trematodes, while in the fish trematode, the effect of insulin was found to be statistically insignificant. The oxygen consumption of G. explanatum and G. crumenifer increased by 19.4% and 11.0% respectively. Islam (1974) reported enhanced glucose uptake in the two mammalian trematodes by 60% in G. explanatum and 27% in G. crumenifer.

From the data available, it is evident that there are conflicting reports about the role of insulin in the carbohydrate metabolism of trematodes. Though the present work does not deal with carbohydrate metabolism of the worms as such, the important relationship between carbohydrate metabolism and gaseous exchange is obvious. Houssay (1959) pointed out that generally insulin increases the uptake of glucose by the cells and its conversion to fat or protein or glycogen or into carbon dioxide and water. Glycogen depletion was noticed in the presence of insulin in F. hepatica by Pantelouris (1964), though later Pantelouris (1965) also stated that uptake of glucose C^{14} was unaffected by insulin. On the other hand Sekardi (1966) reported enhanced glucose uptake by F. hepatica, even during short term incubation with insulin. Isseroff and Read (1968) presented entirely different conclusion. They are of the opinion that insulin has no effect at all on carbohydrate metabolism in the liver fluke.

The results of the present study and of glucose utilization by Islam (1974) clearly indicate that insulin does have a positive effect on the metabolism of G. explanatum and G. crumenifer. The oxygen uptake of G. explanatum is stimulated nearly twice as much when compared with G. crumenifer. This is probably due to the fact that the former inhabits liver

and the latter lives in the rumen.

The effect of 5-hydroxytryptamine (serotonin) on the metabolism of trematodes have been studied by various workers. The results of the present investigation reveal that 5-HT has a stimulatory effect on the oxygen uptake in both fish and mammalian trematodes. The degree of stimulation (62.3%-71.14%) is the same in all three species under study. Again the fish trematode was found to be less sensitive to 5-HT than the mammalian trematodes. The data on the influence of 5-HT on respiration as a function of time is given in Figs. 10-12.

A number of studies have been made with 5-HT, and the first observation that 5-HT affects the liver fluke was made by Mansour (1957) who reported a stimulating effect on the rhythmical movement of F. hepatica, glucose uptake, and glycolysis (Mansour, 1957 a,b; 1958, 1959, 1962). Later on Mansour and Mansour (1962) reported that 5-HT causes increased glycogenolysis in the absence of glucose, with a resultant increase in lactate, but little rise in fatty acid production (Mansour and Lago, 1958), while Lahoud et al. (1971) found reduced rate of glycolysis with prolonged incubation of parasites, and with shorter incubation period 5-HT causes 47% rise in fatty acid production. Islam (1974) reported a positive increase in glucose uptake in G. explanatum and G. crumenifer in the presence

of serotonin. Pretreatment of liver flukes with 5-HT increases phosphorylase activity and stimulates the formation of cyclic 3'-5'-AMP (Mansour et al., 1960) and both 5-HT and 3'-5'-AMP activate phosphofructokinase activity (Mansour and Mansour 1962). Serotonin also affects schistosomes by causing increased motility and glycolysis (Nimmo-Smith and Raison, 1968; Senft and Hillman, 1973). Bennett et al. (1969) and Chou et al. (1972) reported a high concentration of 5-HT in schistosomes and Mansour and Stone (1970) in F. hepatica. In vitro experiments reveal that 5-HT was taken up by S. mansoni (Bennett and Bueding, 1973).

The data on the effect of 5-HT particularly on the respiration of trematodes is very meagre. Hutton et al. (1972) found increased glucose uptake and the incorporation of labelled carbon into carbondioxide in the liver fluke, while Coles (1970) reported an increased oxygen uptake in schistosomes in the presence of 5-HT. Our results are in agreement with the findings of Hutton et al. (1972) and Coles (1970). Mansour (1957) and Mansour et al. (1960) suggested that 5-HT or a related compound may be humoral transmitter for the peripheral receptors in the liver fluke, and it may have a direct effect on the mechanism concerned with the uptake and utilization of oxygen by these worms. The stimulatory effect of serotonin in the

respiration of trematodes suggests that serotonin has direct or indirect role in the metabolism of trematodes.

Histamine was found to be deterimental to fish and mammalian trematodes under study. The worms become immotile when incubated with histamine. The data on oxygen consumption of trematodes with the passage of time indicate that a sudden decrease in oxygen consumption was noticed in all the three species of trematodes. The importance of histamine has been studied extensively in vertebrates, however, it is still uncertain as to what extent histamine is concerned in affecting any physiological process in trematodes. Although histamine has been reported in a number of parasites (Mettrick and Telford, 1963), it has been suggested that histamine plays an important role in the general process of of growth (Kahlson, 1960; Kahlson et al. 1960). Mettrick and Telford (1963) found large amounts of histamine in Mesocoelium monodi, in which the quantity of histamine is greater than found in most mammalian tissue. This species also possesses an exceedingly high histidine decarboxylase. Further, these authors suggest that it is possible that while some parasitic species can manufacture their own histamine, others are dependent on the host tissue. As far as the effect of histamine on the metabolism is concerned, Hutton et al.

(1972) reported that histamine (10^{-4}M) has no significant effect, while Islam (1974) found that the worms become immotile and dead within half an hour when incubated with histamine (10^{-4}M). In the present study the author is unable to explain the negative effect of histamine on the respiration of trematodes. It was considered worthwhile to examine its effect on the carbohydrate metabolism of trematodes. As a matter of fact one would not expect any stimulating effect of histamine in any organism, since it has been found to have deleterious effect when available in free form at least in higher animals.

In the presence of adrenaline and noradrenaline the oxygen consumption decreases in trematodes, however, the results obtained with adrenaline are statistically significant while those with noradrenaline are not statistically significant. The percent inhibition of oxygen uptake ranges from 27.8% to 38.5%. Mansour (1959), Buist and Schofield (1971) and Hutton et al. (1972), found that adrenaline and noradrenaline have no significant role in the metabolism of F. hepatica. However, noradrenaline has been found to occur in schistosomes (Chou et al. 1972). Rogers and Head (1972) reported that noradrenaline activates adenyl cyclase system to supply energy for early development of nematode parasites. Islam (1974) found that noradrenaline has lesser effect on the glucose uptake than other hormones, which

means that only adrenaline might be playing some role in the uptake of glucose. Pantelouris (1965) also holds the opinion that the amount of radioactivity in tissues of F. hepatica was markedly reduced, when adrenaline was added to the medium containing labelled galactose. The results of the present investigation indicate that adrenaline might affect the oxidation process resulting in reduced oxygen uptake. The function of adrenaline in vertebrates is well known, where it is responsible for the breakdown of glycogen in liver and inhibit glycogen synthesis. Further studies on the effect of adrenaline and noradrenaline on the metabolism of trematodes where oxygen is required will be more worthwhile and fruitful.

It is interesting to note that in the presence of testosteron and progesteron no significant influence is seen on the oxygen consumption of adult trematodes. Sex hormones have been mostly examined in studying the relationship between the host and its parasites. From the data available, there seems little doubt that the sex of the host influences the metabolism of platyhelminth parasites (Aldrich et al. 1954, Daugherty, 1956). Berg (1957) reported that testosteron altered the expected sex ratio in S. mansoni. Moor et al. (1954) also obtained equivocal results with testosteron. However, Robinson (1959) failed to demonstrate any effect of the male sex hormones in the same host-

parasite combination. Hutton et al. (1972) were also unable to demonstrate any significant effect on the metabolism of F. hepatica in the presence of these two sex hormones.

The present study indicates that thyroxine, insulin and 5-HT have stimulatory effect, histamine and adrenaline have depressing effect on the respiration of trematodes, while noradrenaline, testosteron and progesteron have statistically insignificant effect.

It should be noted that a particular hormone used in equal amounts was found to influence oxygen uptake differently in the trematodes under study. Among chemical factors which may be of some significance in the chemical physiology of trematodes, the hormones of their host could be one of them. Direct participation of host hormones as metabolic regulators in the control of metabolism may form a basis for the symbiotic relationship between the host and its parasites (Hutton et al. 1972). Some of the studies mentioned above are clear cut indications that there exists an intricate involvement and relationship between the host hormones and its parasite.

VII. Effect of Ions:

Since adult as well as larval stages of trematodes live in a liquid medium, they face a variety of ionic concentrations. It appeared worthwhile to examine the role of different ions on the respiratory physiology of the trematodes, and to see if all species behave similarly or whether there are any inter-specific differences. The results of the various experiments are given in Tables XIX and XX.

All the various ions used in this study were found to have some stimulatory effect on the respiration of the trematodes although the degree of stimulation under various ions is different. In all the three species, K^+ has maximum while Na^+ has least stimulatory effect on the respiration of trematodes. Three concentrations of K^+ were used and it is obvious from the the results that with the increase in K^+ concentration there is a concomitant increase in trematode oxygen uptake. To some extent the relationship is directly proportional.

Sodium ions have a slightly less stimulating effect on the respiration of trematodes. The maximum stimulation was found in the case of G. explanatum (9.7%) while the fish trematode shows only 5.9% increase in its oxygen uptake. The results of the present investigation indicate that sodium is comparatively

Table - XIX. Summary of results of the effect of various ions on oxygen consumption of trematodes in the presence of glucose

Species/Saline	Potassium			Sodium	Calcium		Magnesium		Phosphate	
	Na Free K High	Na Low K High	Na Low K High	Na High K Free	Ca Free	Ca High	Mg Free	Mg High	PO_4 Free	PO_4 High
Isoparorchis hypselobagri	+39.40	+21.5	+17.61	+5.90	-13.73	+ 9.76	-9.37	+ 5.85	-16.73	+23.7
Gastrothylax crumenifer	+30.34	+19.5	+13.31	+ 7.43	- 6.50	+ 8.67	-0.30	+ 9.28	-15.55	+19.5
Gigantocotyle explanatum	+37.45	+21.5	+18.50	+ 9.78	- 9.72	+11.30	-9.76	+11.72	-24.72	+21.8

Values are percentage change in rate of normal oxygen consumption.

Table - XX. Effect of various ions on the rate of oxygen consumption of trematodes in the presence of glucose

Species	Control	Potassium		Sodium		Calcium		Magnesium		Phosphate	
	Normal Saline	Na Free K High	Na Low K High	Na Low K High	Na High K Free	Ca Free	Ca High	Mg Free	Mg High	PO_4 Free	PO_4 High
Isoparorchis hypselobagri	6.08	8.475	7.387	7.151	6.438	5.245	6.674	5.510	6.436	5.062	7.520
Gastrothylax crumenifer	0.323	0.421	0.386	0.366	0.346	0.302	0.351	0.322	0.353	0.276	0.386
Gigantocotyle explanatum	2.14	2.941	2.60	2.536	2.349	1.932	2.382	1.931	2.391	1.611	2.606

Values are in ulO_2 consumed/mg dry wt/hr

less effective than the potassium ions. The results of the effect of sodium and potassium ions suggest that Na^+ can be replaced by K^+ from the medium while studying the respiration of these trematodes.

The effect of Ca^{++} is quite interesting. Absence of Ca^{++} from the incubating medium results in the decrease of the oxygen uptake while high Ca^{++} content causes stimulation. This shows that Ca^{++} also are effective and have some role in the respiratory metabolism of trematodes. I. hypselobagri is comparatively more sensitive to the Ca^{++} and its absence from the saline results in 13.7% inhibition of its respiration. However, the cattle trematodes, G. crumenifer and G. explanatum are less sensitive where only 6.5% and 9.72% inhibition respectively takes place in the oxygen uptake. On the contrary, the increase in calcium content of the incubating medium stimulates the rate of respiration by 11.3% in G. explanatum, and 9.7% in I. hypselobagri and G. crumenifer.

When magnesium and phosphate ions are absent from the incubating saline, it results in a definite decrease while high magnesium and phosphate produces a significant increase in the oxygen consumption of the trematodes under study. If one looks at the results of individual species, the absence of Mg^{++} from the saline produces least inhibition in the oxygen uptake of

G. crumenifer, while high magnesium content causes 9.3% increase in the metabolic rate of the rumen trematode. I. hypselobagri and G. explanatum behave similarly in the absence of Mg^{++} from the saline, i.e., only about 9% inhibition in the oxygen uptake was noticed. However, higher concentration of Mg^{++} results in 5.8% and 11.7% stimulation respectively in the oxygen uptake of the fish and liver trematodes.

The maximum stimulation in oxygen uptake in the presence of high phosphate took place in the fish trematode. Among the mammalian trematodes, the liver trematode shows greater stimulation and inhibition in the respiratory rate in the presence and absence of phosphate from the saline respectively than the rumen trematode.

The above results clearly indicate that the oxygen consumption of trematodes is influenced by the ionic composition of the surrounding medium. Only one such study has ever been made previously on trematodes by Bueding (1950) who reported that potassium and magnesium ions have a stimulatory effect on the metabolic activity of S. mansoni and lower concentration of magnesium resulted in decreased mortality. Bueding et al. (1947) reported that phosphate stimulates the respiration of S. mansoni, but in scolices of Echinococcus it had an opposite

effect (Agosin et al., 1957). Our results are in agreement with Bueding (1950) and Bueding et al. (1947).

von Brand (1943) while working on the influence of ions on the respiration of larval Rustrongyloides reported that oxygen consumption was stimulated by various ions in the following order:

$$'Na = \text{or slightly} < Mg < Ca = NH_4 < K'$$

If the results of the present study are expressed in this manner the following picture emerges:

I. hypselobagri	$Na = Mg < Ca < PO_4 < K$
G. crumenifer	$Na < Ca < Mg < PO_4 < K$
G. explanatum	$Na < Ca = Mg < PO_4 < K$

This clearly shows that K^+ has the maximum while Na^+ has the least stimulatory effect in the respiration of all three species of trematodes. The stimulating effect of K ions is in agreement with the results reported by other investigators on a variety of organisms (literature in Heilbrunn, 1937). Phosphate ions also influence the respiration to the same extent in all three species.

While working on the effect of various ions on the glucose uptake of G. explanatum and G. crumenifer Islam (1974) reported that it depends upon Na^+ and PO_4 ions. High Na^+ and PO_4 cause increased glucose uptake while in the presence of increased potassium trematodes consume less glucose, and Ca^{++} and Mg^{++} have no significant effect on the glucose utilization.

The present investigation reveals that although the effect of various ions on oxygen uptake was found to be similar, however, quantitatively speaking, the three species of trematodes differ from each other in percent stimulation of oxygen uptake.

VIII. Effect of Osmotic Stress:

Though the effect of osmotic stress on the respiratory exchange has been studied in a number of organisms, it has been studied only once in a digenetic trematode by Bair and Peters (1971). In earlier studies on osmotic regulation of digenetic trematodes (Siddiqi and Lutz, 1966; Siddiqi, et al. 1975), percentage change in body weight was used as a parameter of the osmotic behaviour.

In the present study the author has determined the oxygen uptake in three species of digenetic trematodes which were subjected to osmotic stress (hypotonic and hypertonic salines), so as to examine if the latter has any effect on the respiratory metabolism of the worms.

The results of the effect of osmotic stress on oxygen consumption are summarized in Tables XXI and XXII. Fig. 13 shows the percentage change in oxygen consumption (QO_2) in various salines obtained at the end of a fixed period of one hour and Fig. 14 shows the rate of oxygen consumption in different salines.

It can be seen that the three species do not behave in a similar manner as far as the oxygen uptake under various osmotic embarrassments is concerned. I. hypselobagri is not

Table - XXI. Summary of results of the effect of osmotic stress on oxygen consumption of trematodes

Species	Percent saline with glucose								
	0	25	50	75	100	125	150	175	200
Isoparorchis hypselobagri	-23.70	- 9.73	-3.7	-1.4	0	+17.4	+20.7	-53.5	-63.5
Gastrothylax crumenifer	-43.5	-30.5	-20.4	-13.7	0	+27.3	-18.4	-36.8	-44.5
Gigantocotyle explanatum	0.0	-36.7	-25.7	-20.3	0	+23.7	-31.5	-39.7	-43.7

Values are percent change in rate of normal oxygen consumption

Table - XXII. Effect of osmotic stress on oxygen consumption of trematodes in the presence of glucose (8 mM)

Parasite	% Saline	n	QO_2 Mean	SE	Percent of change in initial rate	p Value	Significance*
Isoparorchis hypselobagri							
	Control	8	6.080	±0.069			
	0	5	4.639	±0.101	-23.70	<0.001	+++
	25	7	5.488	±0.070	- 9.73	<0.02	++
	50	4	5.855	±0.031	- 3.70	=0.05	+
	75	5	5.990	±0.006	- 1.40	<0.5	+
	125	5	7.137	±0.359	+17.40	=0.1	-
	150	4	7.338	±0.363	+20.70	=0.05	+
	175	7	2.827	±0.876	-53.50	<0.05	+
	200	6	2.219	±0.262	-63.50	<0.001	+++
Gigantocotyle explanatum							
	Control	8	2.140	±0.031			
	0	5	Dead				
	25	5	1.354	±0.166	-30.70	<0.02	++
	50	5	1.590	±0.117	-25.70	<0.02	++
	75	5	1.705	±0.149	-20.30	=0.1	-
	125	5	2.647	±0.115	+23.70	<0.05	+
	150	5	1.465	±0.195	-31.50	<0.05	+
	175	5	1.290	±0.214	-39.70	<0.025	++
	200	5	1.211	±0.203	-43.40	<0.02	++
Gastrothylax crumenifer							
	Control	8	0.323	±0.004			
	0	7	0.182	±0.019	-43.65	<0.001	+++
	25	6	0.224	±0.029	-30.65	=0.05	+
	50	7	0.257	±0.031	-20.40	=0.05	+
	75	4	0.278	±0.059	-13.93	<0.5	-
	125	5	0.411	±0.073	+27.24	=0.05	+
	150	7	0.263	±0.154	-18.57	<0.05	+
	175	6	0.204	±0.366	-36.84	=0.05	+
	200	7	0.179	±0.301	-44.58	<0.02	++

* - Insignificant, + Significant, ++ Highly significant, +++ Very highly significant

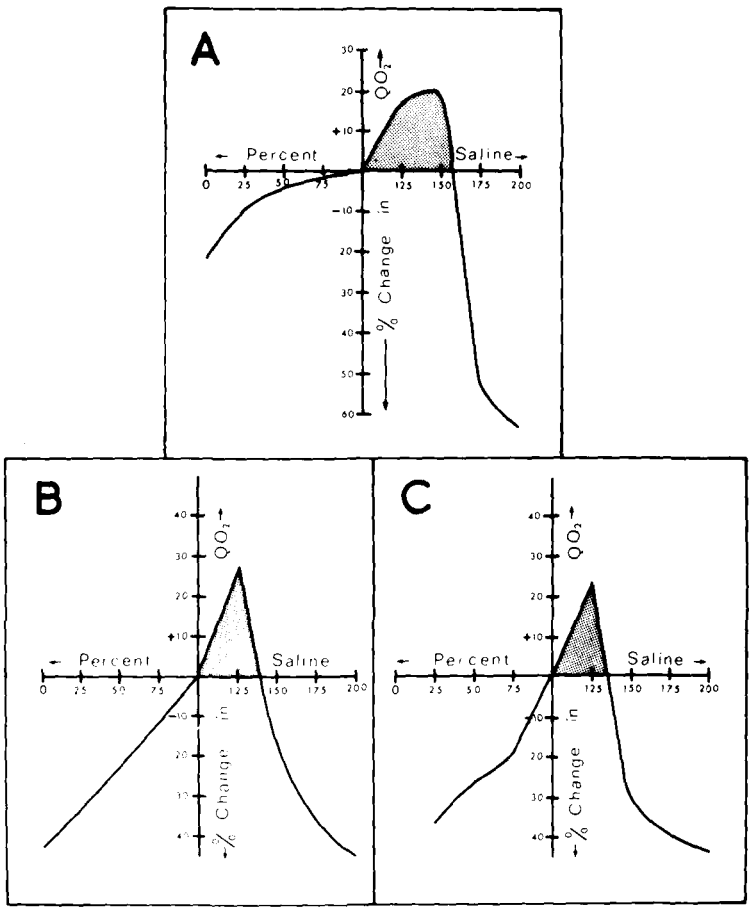


Fig. 13. Percentage change in oxygen consumption of trematodes in various concentrations of salines.

A. Isoparorchis hypselobagri.

B. Gastrothylax crumenifer.

C. Gigantocotyle explanatum.

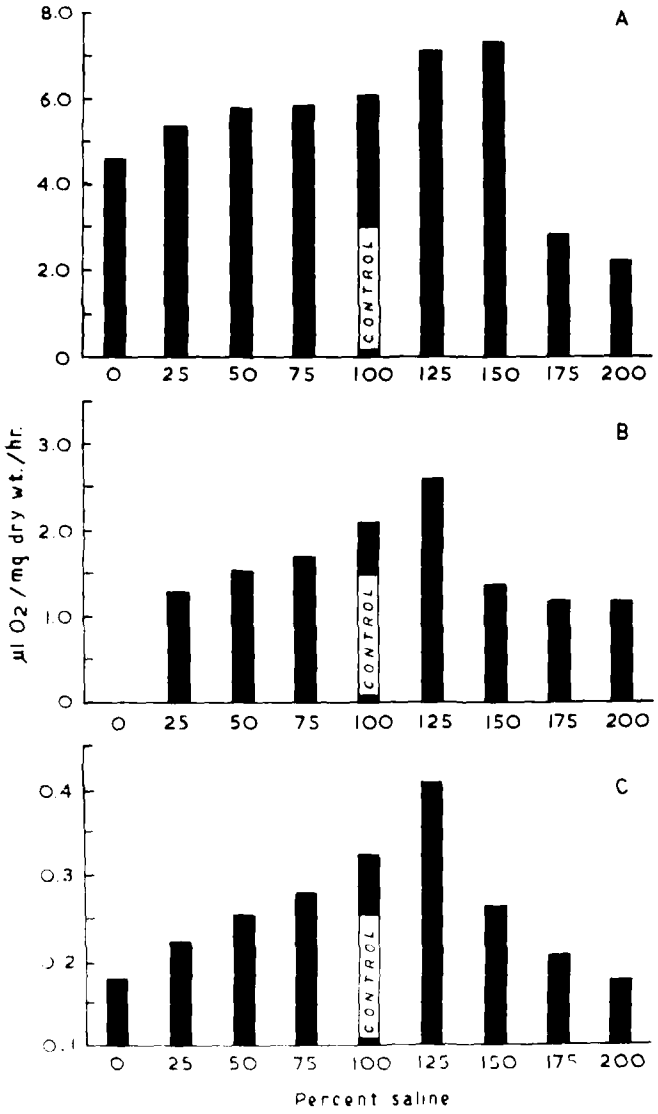


Fig. 14. Rate of oxygen consumption of trematodes in various concentrations of salines.

A. I. hyoselobarri.

B. G. explanatum.

C. G. crumenifer.

sensitive to hypotonic solutions as much as are the rumen and the liver flukes. Among the mammalian trematodes, the depression in oxygen uptake in G. crumenifer, is more or less similar to G. explanatum except that the latter becomes water logged and dies in deionized water. The hypertonicity beyond 135% is deleterious to the mammalian trematodes, and beyond 150% to the catfish trematode. Hypertonicities above 150% are highly inimical to the oxygen uptake in I. hyderabagri than in the mammalian trematode, i.e., the depression in oxygen uptake in I. hyderabagri is 60% compared with 40% in the case of mammalian flukes.

From the results described above one can see that both hypotonicities as well as extreme hypertonicities (beyond 150%) are inimical to the oxygen uptake in the three species. In the case of I. hyderabagri the hypotonic solutions are less harmful and the extent of depression in oxygen consumption is much less compared with the hypertonic media where nearly three times less oxygen is consumed than in deionized water (Table XXII). G. crumenifer is equally sensitive to hypo- and hypertonic media and the extent of depression in oxygen uptake is similar in both cases. G. explanatum is very sensitive to deionized water, otherwise its behaviour and the extent of oxygen consumption is identical to G. crumenifer. It can also be seen from Fig. 13

and 14 and Tables XXI and XXII that in 125% though oxygen uptake goes up it is statistically insignificant in the case of I. hynselobagri ($P = .1$) and just significant in the case of G. crumenifer and G. explanatum ($P < 0.05$). I. hynselobagri is less sensitive to hypotonic solutions and therefore the extent of decrease in oxygen uptake is not very great, but it is very sensitive to extremely hypertonic solutions (i.e., beyond 155%) and as a result the decrease in QO_2 is very highly significant ($P < 0.001$ in 200%).

IX. Effect of Carbon Monoxide:

For the present study, three trematode species were chosen: I. hypselobagri, G. crumenifer and G. explanatum. The results are summarized in Table XXIII.

The results reveal that in all the three species under study CO produces a depressing effect on their rate of respiration. I. hypselobagri is comparatively more sensitive to carbon monoxide than the mammalian trematodes. Among the latter, the respiratory rate decreases more in G. explanatum than G. crumenifer. However, in all cases the extent of inhibition increases with the increase in incubation time of the parasite with carbon monoxide, although individual differences in the percent inhibition in respiration may be due to the species differences. The maximum inhibition in fish trematode is probably due to the fact that this parasite lives in an oxygen rich environment, whereas liver and rumen trematodes of mammals live in habitats where oxygen tension is very low. Similar results have been reported by van Grembergen (1949) while studying oxygen uptake in F. hepatica after CO treatment.

Carbon monoxide combines with haemoglobin even more rapidly than does oxygen. It has been mentioned earlier that the trematodes under study possess haemoglobin. Haider (1975) reported that CO readily combines with trematode haemoglobin and the

Table - XXIII. Effect of carbon monoxide on oxygen uptake of trematodes in presence of glucose (8 mM)

	n	Time of incubation with CO (min)	QO_2	Percent of inhibition in initial rate	p Value	Significance*
Isoparorchis hypselobagri						
Control	4	--	5.930± 0.071			
	3	20	4.862± 0.634	-18.0	< 0.05	+
	5	40	3.202± 0.452	-46.3	< 0.025	++
	5	60	1.215± 0.018	-79.5	< 0.001	+++
Gigantocotyle explanatum						
Control	5	--	2.109± 0.020			
	3	20	1.934± 0.017	- 7.8	< 0.025	++
	3	40	1.299± 0.212	-38.4	< 0.05	+
	3	60	0.765± 0.260	-63.7	=0.01	++
Gastrothylax crumenifer						
Control	5	--	0.331± 0.003			
	5	20	0.278± 0.011	-15.9	< 0.05	+
	3	40	0.201± 0.025	-39.18	< 0.02	++
	5	60	0.133± 0.043	-59.7	<0.02	++

* - Insignificant, + Significant, ++ Highly significant, +++ Very highly significant

resultant carboxyhaemoglobin shows characteristic and specific absorption maxima. The depression in the respiratory rate of the three trematodes as a result of CO treatment throws light on the fact that the trematode haemoglobin may be playing some role in oxygen transport. Besides, a functional but slightly modified cytochrome system, cytochrome oxidases and reductases also exist in trematodes (Buedin; and Charms, 1952; deZoeten and Tipker, 1969; Prichard and Schofield, 1968, 1971; Coles 1972, Cheah and Prichard, 1974). Cheah (1967) and Prichard (1974) reported that fumarate reductase action appears to involve a cytochrome-containing system of electron transport. Cheah (1972) suggested that cytochromes also appear to be involved in trematode respiration. However, the cytochrome chain is not identical with that found in vertebrate tissue as O-type cytochrome oxidases appear to be quantitatively more significant than cytochrome aa_3 (Kurelec, 1975).

In view of the above facts it is evident that carbon monoxide must have some effect on the respiratory metabolism, because in carbon monoxide poisoning some of the iron atoms of the haemoglobin molecule hold carbon monoxide and ultimately interfere in the terminal cytochrome system. Carbon monoxide poisioning takes place only in aerobic animals. From the present study one can conclude that trematodes also to some extent can be considered to be facultatively aerobic animals.

The results of the present study provide an indirect evidence that haemoglobin is playing some role in oxygen transport and a cytochrome system might be functional in trematodes under study. However, further research on the terminal respiratory metabolism of trematodes is required.

X. Effect of Anaerobic Incubation:

The accumulation of oxygen debt has been studied in many helminths but the trematodes have remained more or less neglected. The only species living in an oxygen rich environment is Paramphimus sp., that has been studied for this purpose (Read and Yogore, 1955 and Bruce et al., 1971). Among the four species under study, I. hyderobagri is a trematode which appears to be aerobic in nature and lives in an oxygen rich habitat (Siddiqi and Nizami, 1974, 1975).

The worms were subjected to varying periods (30, 60, 90, and 120 minutes) of incubation under anaerobic conditions. The oxygen consumption was measured at 20 minutes interval to examine the effects of anaerobic incubation. Simultaneously normal controls were run and the results are shown in Table XXIV and Fig. 15.

It is quite evident that as a result of anaerobic incubation more oxygen is consumed in the beginning than at the end of the experiment. With the increase in the period of anaerobic incubation there is a corresponding increase in the oxygen consumption by the trematode. In other words the respiratory overshoot is proportional to the period of anaerobic incubation. However, the increase in respiration does not last very long and tends to return to normal after the first 20 minutes. The negative values in the percentage change in respiration are

Table - XXIV. Effect of anaerobic incubation on the respiration of Isoparorchis hypselobagri, in the presence of glucose (8 mM)

Anaerobic Incubation Time = 30 min

Time (min)	n	QO_2		Percent change in initial rate
		Control	Post-anaerobic	
20	4	2.215	2.437	+10.02
40	6	1.851	2.497	+34.90
60	4	1.871	2.371	+26.17
80	5	1.735	2.099	+20.86
100	3	2.385	1.953	-18.11
120	5	1.935	1.883	- 5.01

Anaerobic Incubation Time = 60 min

Time (min)	n	QO_2		Percent change in initial rate
		Control	Post-anaerobic	
20	6	1.931	2.851	+47.64
40	5	2.172	2.731	+25.73
60	5	2.101	2.495	+18.75
80	3	2.181	2.375	+ 8.90
100	8	1.993	1.759	-11.74
120	6	1.995	2.057	+ 3.10

Continued

Table - XXIV (Contimued)

Anaerobic Incubation Time = 90 min

Time (min)	n	QO_2		Percent change in initial rate
		Control	Post-anaerobic	
20	4	2.317	3.451	+48.94
40	5	2.158	3.170	+46.90
60	3	2.109	2.975	+86.60
80	3	2.005	2.458	+22.60
100	4	1.997	2.355	+17.92
120	3	1.985	2.095	+ 5.54

Anaerobic Incubation Time = 120 min

Time (min)	n	QO_2		Percent change in initial rate
		Control	Post-anaerobic	
20	5	2.175	4.230	+ 49.48
40	3	1.990	4.095	+105.78
60	3	1.887	3.581	+ 89.76
80	4	1.773	2.615	+ 47.49
100	5	1.780	2.491	+ 39.94
120	3	1.895	2.631	+ 38.84

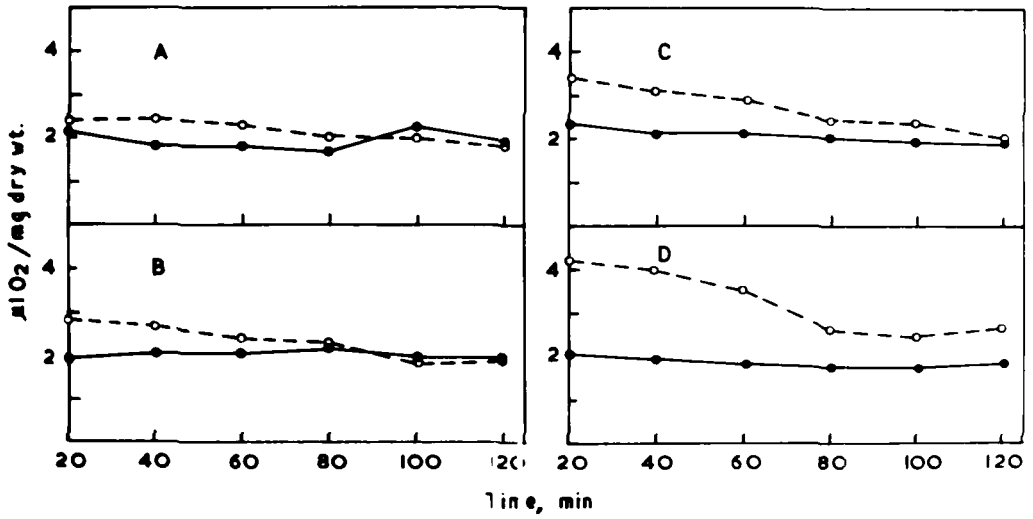


Fig. 15. Effect of anaerobic incubation on the respiration of I. hypselobagri.

A. 30 minutes anaerobic incubation

B. 60 minutes anaerobic incubation

C. 90 minutes anaerobic incubation

D. 120 minutes anaerobic incubation

—— CONTROL

- - - AFTER INCUBATION

statistically insignificant.

The above results clearly indicate that anoxic incubation results in the accumulation of an oxygen debt and a process of repayment of oxygen exists in I. hypseloba ri. The degree of repayment of oxygen seems to be dependent upon the period of anoxic incubation, i.e., longer the period of anoxia hi her the respiratory overshoot.

Among trematodes, Schistosoma mansoni (Buedin;, 1950) and Gynaecotyle adunca (Hunter and Vernberg, 1955) show no evidence of accumulatin; an oxygen debt durin; periods of anoxia. However, in the case of S. mansoni, complete excretion of the fatty acid intermediates have been reported. Whereas, uptil now only a few species of trematodes have been shown to pay oxygen debt; these are P. ohirai (Bruce et al. 1971) and P. westermani (Read and Yogore, 1955). The most appropriate explanation for the failure in some trematodes to repay oxygen debt is that, such a debt does not develop due to the metabolic characteristics of these parasites in habitat. von Brand (1938) pointed cut that the oxygen content of some habitats like intestine or bile duct is extremely low and the parasite of such habitats are generally highly resistant to hypoxia and obtain their energy via anaerobic processes. Hymen (1951) suggested that the respiratory metabolism of F. hepatica

is one of anaerobic type although it would consume oxygen when it is available. This oxygen appears to have limited metabolic usage since the CO_2 produced remains constant regardless of the amount of oxygen consumed. While in F. hepatica; the presence of oxygen did not significantly affect the use of carbohydrates or the nature of the organic acids which were excreted (Mansour, 1959).

Generally, the phenomenon of oxygen overshoot is due to the accumulation of end products of anaerobic metabolism. The increase in substrates would lead to an increased probability of enzyme and substrate molecules colliding (Zimmerman, 1949). The period of repayment seems to indicate that a regulatory mechanism exists in the trematodes which releases the accumulated substrates only slowly to the oxidative process as von Brand (1953) suggested in the case of snails. Later von Brand (1973) pointed out that the mechanism by which the surplus oxygen acquired by the animal to detoxify the end products of anaerobic incubation either by oxidation or by resynthesis to carbohydrates shows a dependency of their respiration of the oxygen tension.

It may be possible that in trematodes due to anaerobic incubation, the lactic or pyruvic dehydrogenases may be affected. Because LDH is required for the interconversion of lactic and

pyruvic acids, while pyruvic dehydrogenase is required for the formation of acetyl CoA.

Baeyens et al.(1974) found that LDH activity of various tissues of frog and dog is reduced when exposed to hyperbaric oxygen. Bryant (1971) pointed out that "the biochemical key to the problem of the switch from aerobic to anaerobic metabolism must lie with the enzyme responsible for the interconversion of pyruvic and lactic acids. The nature of the enzyme responsible, lactic dehydrogenase, is still not clearly understood, but it is becoming more apparent that one of its role is to assist metabolic regulation."

The above idea seems to be worthwhile. Lactic dehydrogenase and its isozymes in trematodes have been reported by a number of workers, and further research on the mechanism of oxygen debt and its affinity with enzymes and substrates will help us in understanding the actual mechanism involved for the overshoot of oxygen uptake in trematodes after a period of anaerobic incubation.

XI. Effect of Substrates of the Tricarboxylic Acid Cycle:

The tricarboxylic acid cycle has been proved to be the major oxidative pathway in many groups of animals, but among trematodes the role of this cycle is disputed. As a result of the present study it was concluded beyond doubt that I. hypselobagri is an example of an aerobic trematode, and the present experiment was carriedout so as to determine whether the TCA cycle is operative in this trematode.

When intermediates of the TCA cycle were added to the fish saline, an increase in the respiration rate was noticed in the case of all substrates tested. The results are summarized in Table XXV. The maximum stimulation of the oxygen uptake was noticed in the presence of succinate while least stimulation was produced by citrate and ∝-ketoglutarate. The extent of stimulation of xygen consumption in the case of each substrate seems to be dependent upon the concentration of the substrate; as the concentration increases so does the oxygen consumption.

Various enzymes of the TCA cycle have been reported by a number of workers in various trematodes, D. dendriticum (Kohler and Hanselmann, 1973), F. hepatica (Pennoit-DeCooman and van Grenbergen, 1942; Prichard and Schofield, 1968;

Table - XXV. Effect of substrates of the TCA cycle on the oxygen consumption of Isoparorchis hypselobagri at 30°C

Substrates	Concentration	n	Q_{O_2} Mean	SE	% Change	p Value	Significance *
Control		7	4.371	±0.037			
Citrate	.01M	5	4.917	±0.171	+12.5	<0.05	+
	.015M	6	5.214	±0.236	+19.3	<0.05	+
Isocitrate	.005	6	4.382	±0.144	+11.7	<0.05	+
	.01M	6	5.214	±0.226	+19.3	<0.025	++
	.015M	4	5.616	±0.312	+28.5	<0.02	++
-ketoglutrate	.005M	4	4.751	±0.102	+ 6.7	<0.025	++
	.01M	5	4.965	±0.176	+13.6	<0.05	+
	.015M	5	5.227	±0.237	+19.6	<0.025	++
Succinate	.01M	4	5.564	±0.171	+27.3	<0.001	+++
	.05M	5	6.184	±0.319	+41.5	<0.005	++
	.1M	4	7.356	±0.333	+68.3	<0.001	+++
Fumarate	.005M	3	4.974	±0.174	+13.8	<0.025	++
	.015	5	5.262	±0.270	+20.4	<0.005	++
Malate	.005M	5	4.978	±0.161	+13.9	<0.025	++
	.01M	5	5.319	±0.235	+21.7	<0.02	++
	.015M	4	6.106	±0.418	+39.7	=0.01	++
Oxaloacetate	.01M	7	5.568	±0.109	+27.4	<0.001	++
	.015M	5	6.718	±0.428	+53.8	<0.005	+

* - Insignificant, + Significant, ++ Highly significant, +++ Very highly significant

de Zoeten et al. 1969), S. japonicum (Huang and Chu, 1962), S. mansoni (Conde-del Pino et al., 1968 and Coles, 1971, 1972). Besides, various intermediates have been reported in adult trematodes, F. hepatica (Bryant and Williams, 1962; Bryant and Smith, 1963; Thorsell, 1963) and P. westermani (Hamajima, 1967). This indicates that the TCA cycle exists in various trematodes although its role in the metabolism is controversial. Vernberg and Hunter (1960) reported that the respiratory rate of G. adunca, (a parasite of small intestine) increases in the presence of succinate, malate and oxaloacetate; while citrate, isocitrate, and pyruvate was found to be ineffective. In F. hepatica the oxygen consumption increases in the presence of succinate and malate (van Grembergen, 1949). Recently, Hamajima (1972) reported high oxygen uptake in adult Paragonimus miyazakii (lung parasite) in the presence of pyruvate, citrate, cisaconitate, isocitrate, α-ketoglutarate, succinate, fumarate, malate and a mixture of oxaloacetate and pyruvate. He also reported that succinate showed the strongest effect on oxygen uptake, being almost three times as much as that of endogenous respiration and least effect was seen in the presence of citrate and α-ketoglutarate. The results of the present investigation support and confirm the effect of succinate citrate and α-ketoglutarate.

The functional role of the TCA cycle in trematode metabolism is one of the controversial aspects of the biochemistry and physiology of these parasites. von Brand (1973) exhaustively reviewed the literature on the Krebs cycle in parasites and pointed out that many helminths either have no Krebs cycle at all or it is active at such low levels that its functioning cannot be established unambiguously. Although all the enzymes of the TCA cycle are present and there is active citrate synthase, the low activity of aconitase and isocitrate dehydrogenase suggests that the cycle is of only a minor importance (Prichard and Schofield, 1968; Sturm et al., 1969). Various enzymes of this cycle have been reported in D. dendriticum, but only very small amounts of α-ketoglutarate dehydrogenase were detected, suggesting that citric acid cycle is not important. This conclusion is supported by Kohler and Hanselmann (1973). However, Prichard and Schofield (1969) reported the production of glyoxalate as well as the formation of labelled malate from the labelled glyoxylate plus acetyl CoA suggests that a functional glyoxylate cycle is present in F. hepatica.

On the other hand, Coles (1973) suggests that there is some evidence for a functional citric acid cycle in Schistosomes on the basis of the presence of enzymes and inhibitory studies. Flouroacetate partially inhibits oxygen uptake (Coles, 1970) and

various enzymes have been demonstrated, citrate synthese, isocitrate dehydrogenase (Coles, 1972) succinic oxidase (Smithers et al., 1965; Coles, 1973) and malate dehydrogenase (Conde del-Pino et al., 1966, Buedin; and Saz, 1968, Coles, 1970, 1971, 1973). While Tada et al. (1961) and Hamajima (1967, 1972) have reported that a functional TCA cycle seems to be present in adults of P. westermani and P. miyazakii. In the present experiments similar results were obtained by using oxygen uptake as a parameter in the presence of various substrates in adult I. hypselobagri. On the contrary, Ward and Fairbairn (1970) found that the TCA cycle is not operative in Hymenolepis diminuta. Besides, Oya et al. (1965) and Prichard and Schofield (1968) reported that this cycle may be of minor importance in Ascaris muscle and in adult F. hepatica.

The results of the present investigation provide indirect evidence in support of a TCA cycle being present in I. hypselobagri. Only further research on the enzymesof the TCA cycle will elucidate the metabolic pathways in this digenetic trematode.

C H A P T E R - VI

SUMMARY AND CONCLUSIONS

The results obtained on various aspects of respiratory metabolism of four species of trematodes, Isoparorchis hypselobagri from the swim bladder of the catfish, Wallago attu, Cotylophoron cotylophorum, Gastrothylax crumenifer from the rumen and Gigantocotyle explanatum from the liver of the Indian water buffalo, Bubalus bubalis, have been described in the present dissertation.

It was found that I. hypselobagri consumes more oxygen than the mammalian trematodes. Among the cattle trematodes G. explanatum consumes more oxygen than the rumen trematodes; while among the rumen trematodes G. crumenifer consumed more oxygen than C. cotylophoron. The differences in the normal oxygen consumption of trematodes is probably due to the water content of the parasite, high and low oxygen tensions in their respective habitats and also due to the species differences. The significance of oxygen consumption in the metabolism of trematodes is difficult to explain at the present stage. Further research on this aspect is required.

The optimum temperature for oxygen consumption is different in fish and mammalian trematodes, i.e., 30° and 40°C respectively. The metabolism of the fish parasite continues to be normal at lower temperatures while higher temperature (45°C) causes sudden decrease in oxygen uptake. In the mammalian trematodes, lower temperatures, drastically retard the metabolic activity. The different temperature optima for the fish and mammalian trematodes are probably due to the fact that the former is a parasite of a poikilothermic animal whereas the latter live in a homeothermic animal. The results of the present investigation reveal that the metabolic temperature response of these trematodes closely parallel the body temperature of their respective hosts. Such studies would be interesting to examine similarities and differences between the parasites of poikilothermic and homeothermic animals, which might exist as a consequence of niche specialization.

The pH has pronounced effect on the respiratory rate of these trematodes. There is an optimum range over which the oxygen consumption of the fish, rumen and liver trematodes remain more or less unaltered. It is evident from the present study that the rumen trematodes show a wider range of pH in which their rate of respiration remains unchanged. Similarly

the liver trematode shows maximum oxygen uptake in an alkaline range. It can be concluded from the present investigation that the respiration of parasites depends upon the pH of the surrounding medium or microenvironment in which these parasites live, and support the fact that the nature of the habitat has influenced the biochemical and physiological characteristics of the parasites living in that habitat.

Various hexoses, disaccharides, pentose sugar, amino acids and other substrates were provided into the medium to examine not only their effect on the respiratory rate but also to see if there is any particular food preferences on the part of trematodes under study. In all the four species of trematodes, the effect of various substrates except maltose was found to be stimulatory; though the extent of stimulation of oxygen consumption in each species is different. If oxygen uptake is considered as a parameter of hexose utilization than glucose and fructose were found to be more stimulatory and easily utilized than amino acids in all the four species. The maximum stimulation in respiration was noticed in the mammalian trematodes in the presence of glycerol, whereas in the fish trematode, glucose causes maximum stimulation. It can be concluded from the present investigations that all the four species make use of carbohydrates, amino acids and other substrates, but

they exhibit species differences and are adapted to utilizing one substrate better than others. However, C. cotylophorum and G. crumenifer appear to be quite similar as far as the utilization of sugars is concerned when compared with I. hypselobagri and G. explanatum. These findings suggest that these substrates are readily taken up and utilized as energy source in the metabolism of trematodes, and also one can safely conclude that the trematodes have food preferences.

A number of chemicals were tested for their inhibitory or stimulatory effect on the oxygen consumption of these trematodes. It was noticed that all the chemicals used in this experiment except 2,4-dinitrophenol and 2,4-dinitrocyclopentyl phenol were found to act as "inhibitor" though some are better and more effective than others, as can be seen by the extent of percentage change in the oxygen uptake. It is interesting to note that some chemicals used for the four species in equal concentration were found to be similar in action in all the four species of trematodes and the extent of inhibition or stimulation is more or less of the same order with few exceptions. Among the chemicals tested, potassium cyanide was found to be more inhibitory in all the four species, while diethyldithiocarbamate was found to be least effective in trematodes under investigation. Ethylurethane, salicylaldoxime, sodium arsenite,

2,4-dinitrophenol, 2,4-dinitrocyclopentyl phenol, iodoacetate, malonate, potassium cyanide, p-chloromercuric benzoate were found to be more effective inhibitors or stimulators of respiration in the fish trematodes than in the mammalian trematodes.

The degree of stimulation or inhibition of oxygen consumption caused by various chemical compounds is probably dependent upon differential permeability of the tegument of trematodes which appears to be influenced by parasitism in different habitats. The inhibitory studies on oxygen consumption in the presence of various compounds provides an indirect evidence about the different metabolic pathways in trematodes, these compounds inhibit the activity of various enzymes which results in the retardation of oxygen uptake.

Such studies help in understanding the host-parasite relationships, nature of the metabolism of the trematodes and also in the chemotherapeutic treatment of parasitic diseases.

The effect of various hormones on the respiration of trematodes indicates that they inhibit or stimulate the oxygen uptake with few exceptions. Thyroxine, and 5-HT have a stimulatory effect; histamine and adrenaline have depressing effect on the respiration of trematodes, while noradrenaline, testosteron, and progesteron have statistically insignificant effect.

The extent of inhibition or stimulation is more or less of the same order with the exception of insulin in the case of G. explanatum, which consumed more oxygen in the presence of insulin. This stimulatory effect of insulin on the liver parasite is due to the fact, that being a parasite of liver it may be more sensitive to the action of insulin than other parasites under study.

It should be noted that a particular hormone used in equal concentration was found to influence oxygen uptake differently as far as the degree of stimulation or inhibition is concerned in the trematodes under study. Such type of studies form a basis for the symbiotic relationship between the host and its parasites.

Na^{++}, K^{+}, Ca^{++} and PO_4 ions have a pronounced stimulatory effect on the respiration, although the degree of stimulation by the various ions is different. In all the three species under study K^{+} has maximum while Na^{+} has least stimulatory effect on the respiration of trematodes. The degree of stimulation of different ions on the oxygen uptake in three species of trematodes under study can be expressed as follows:

I. hypselobagri	$Na = Mg < Ca < PO_4 < K$
G. crumenifer	$Na < Ca < Mg < PO_4 < K$
G. explanatum	$Na < Ca = Mg < PO_4 < K$

The present investi ation reveals that although the effect of various ions on oxygen uptake was found to be similar, however, quantitatively speaking, the three species of trematodes differ slightly from each other in percent stimulation of oxygen uptake. Such differences are due to the influence of their habitats.

It can be concluded from the present study that Na^{+}, K^{+}, Ca^{+}, Mg^{++} and PO_4 ions are important ingredients of the saline, which are required for the optimum respiration of the trematodes. Necessary changes can be made in the incubating media and physiological salines in the light of the results of the present studies.

The results of the osmotic studies clearly show that both hypotonic and extreme hypertonic salines are inimical to the oxygen uptake in all the three species under study. However, catfish trematode is less sensitive to hypotonicities than extreme hypertonicities. The mammalian species are equally sensitive to hypo- and hypertonic media and the extent of depression in oxygen consumption is similar in both species.

Carbon monoxide causes a depressing effect on the respiration of trematodes. I. hypselobagri is comparatively more sensitive to carbon monoxide than the mammalian trematodes.

Among the latter the QO_2 decreases more in G. explanatum than G. crumenifer. In all the species under study, inhibition of oxygen uptake is directly proportional to the incubation time of the trematodes with carbon monoxide. The maximum inhibition in the fish trematode is probably due to fact that this parasite lives in an oxygen rich environment, whereas the liver and the rumen trematodes live in oxygen poor habitats.

The trematode haemoglobin may be playing some role in oxygen transport, and a cytochrome system might be functional in trematodes under study. It can be concluded from the present study that trematodes also to some extent can be considered to be facultatively aerobic animals.

As a result of anaerobic incubation, I. hypselobagri develops oxygen debt which results in a respiratory overshoot when exposed to air and there exists a direct relationship between the period of anaerobic incubation and the respiratory overshoot. However, the increase in respiration tends to return to normal after the first 20 minutes.

The above results clearly indicate that anoxic incubation results in the accumulation of an oxygen debt and a process of repayment of oxygen debt exists in I. hypselobagri, although the degree of oxygen debt seems to be dependent upon the period of anoxic incubation. The present study also

provides an indication that I. hypselobagri may possess a mechanism by which it can live both aerobically as well as anaerobically.

It has been observed that the intermediates of the TCA cycle (oxaloacetate, citrate, isocitrate, α-ketoglutarate, succinate, fumarate and malate) significantly stimulate the respiration in I. hypselobagri. The maximum stimulation was noticed in the presence of succinate, while least stimulation was produced by citrate and α-ketoglutarate. The extent of stimulation of oxygen uptake in the case of each substrate seems to be dependent upon the concentration of the substrate; as the concentration increases so does the oxygen consumption.

The results of the present study provides an indirect evidence in support of a TCA cycle being present in I. hypselobagri. Increased oxygen uptake in the presence of various substrates points to the presence of various enzymes of TCA cycle in this parasite.

As a result of the present investigations it can be safely concluded that I. hypselobagri is a facultative aerobic trematode while the other species, G. explanatum, G. crumenifer and C. cotylophorum appear to be facultative anaerobic trematodes. The various experiments that were carried out, provide an insight into the physiology of this important group of parasites and will help a long way in devising amiable physiological in vitro conditions for further research.

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P U B L I C A T I O N S

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Studies on *in vitro* Survival of *Isoparorchis hypselobagri* (Digenea: Trematoda)

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Summary. *Isoparorchis hypselobagri* survives longer under aerobic rather than under anaerobic conditions in a balanced salt solution containing glucose, although it continues to loose weight; when the blood was added to the medium, the rate of weight loss of the parasite was reduced to half of that in glucose saline, with greater production of hematin. Mature worms continued to lay eggs during the first ten days after which the egg production deteriorated. Metabolites of the parasite lowers the pH of the medium. The constituents of the medium are still not optimal, which results in gradual deterioration of worms. A synthetic or chemically defined medium should be devised for *in vitro* culture of this trematode.

Introduction

The digenetic trematodes live in a variety of habitats and both adults and larval stages of a number of species have been used for *in vitro* culture studies. Literature on the subject has been reviewed by Silverman (1965); Berntzen (1966); Clegg and Smyth (1967) and more recently and exhaustively by Taylor and Baker (1968).

Any trematode whose microenvironment is relatively less complicated would be a good tool for *in vitro* culture studies than species living in complex and poorly understood habitats. *Isoparorchis hypselobagri* is very commonly parasitic in the swim bladder of a freshwater fish, *Wallago attu* in Aligarh and provides a unique opportunity to study some aspects of trematode physiology. The swim bladder is a simple but not much explored biological niche, where blood, whose composition is known, is the only source of nutrition for the trematode parasite and where biologically significant amount of oxygen is present (Siddiqi and Nizami, 1974). In view of these facts, it was presumed that the requirements of this trematode could be very complex and that it could be easily cultured *in vitro*.

The purpose of this study was to make exploratory observations on the survival of *I. hypselobagri* in simple culture media under aerobic and anaerobic conditions.

Materials and Methods

The worms were collected from the swim bladders of *Wallago attu* which were obtained from the local fish market. The worms were washed three times with sterile culture medium, damp dried on a Whatman filter paper No. 1 and the initial wet weight of parasites was recorded on a single-pan balance. Only active flukes were used for *in vitro* culture experiments. For aerobic cultivation 50 ml Erlenmeyer flasks with cotton plugs were used and for anaerobic studies, 100 ml Erlenmeyer flasks with a rubber stopper with two stopcocks were used; one for gas-inlet and the other for gas-outlet. The glassware was acid cleaned and finally washed with double destilled water. All glassware and instruments were sterilized under 15 lb p.s.i. pressure for 30 minutes.

The medium in which the parasites were maintained for *in vitro* culture, contained NaCl 100 mM, KCl 2.5 mM, $CaCl_2$ 1.5 mM, $MgCl_2$ 1.0 mM, NaH_2PO_4 0.5 mM, $NaHCO_3$ 5.0 mM and glucose 19.9 mM. The pH of the medium was adjusted to 7.0. For the maintenance of axenic conditions, penicilline G (Na Salt) 100 units/ml, streptomycine sulphate 100 µg/ml and mycostatin 20 ppm were added to the medium. Sintered or fritted glass and asbestos filters (Seitz type) were used for sterilization of the medium in order to avoid bacterial and fungal contamination.

Only one worm was placed in a culture flask containing 20 ml sterile medium and the culture medium was changed on every alternate day to avoid bacterial or fungal growth, and every 10th day the weight of the parasite was recorded. Anaerobic conditions were established by using nitrogen as the gas phase. Both the stopcocks of the culture flask were kept open in order to replace air by the nitrogen gas. The nitrogen gas was allowed to bubble into the medium for about 5 minutes, and the outlet stopcocks were closed first, immediately followed by the closure of the gas-inlet stopcocks. In anaerobic experiments, whenever the culture medium was changed, air was replaced with nitrogen in the manner mentioned above.

In some experiments, 5 ml bovine blood was also added to the above medium to see if the parasites fared better in its presence. For this study, ACD (acid-citrate-dextrose) solution was used as an anticoagulent for bovine blood. The flasks were swirled frequently to disperse the waste products more evenly throughout the solution.

Results and Discussion

The criteria of normal survival used in these experiments were quadrifold: weight, movement, and colour of the parasite, and changes in the pH of the culture medium. These preliminary *in vitro* culture studies revealed that on the 10th, 20th, 30th, and 40th day, the survival of *Isoparorchis hypselobagri* under aerobic conditions was 100%, 100%, 84% and 44% respectively; while under anaerobic conditions it was only 80%, 83%, 1% and 0% respectively. Under aerobic conditions the maximum survival was for 49 days whereas under anaerobic conditions it was only for 30 days (Fig. 1).

The weight of the parasite was used as a parameter of successful *in vitro* culture. In worms under anaerobic experiments the weight loss was higher compared with aerobic experiments, but on adding blood to the medium the extent of weight loss was reduced almost to half

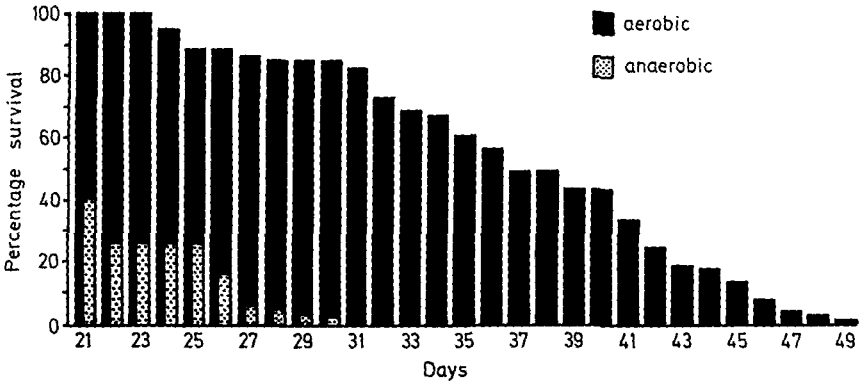

Fig. 1. *In vitro* survival of *Isoparorchis hypselobagri* under aerobic and anaerobic conditions

Table 1. Percentage weight loss of *Isoparorchis hypselobagri* under aerobic and anaerobic conditions

Days	With blood		Without blood	
	Aerobic (41)	Anaerobic (33)	Aerobic (56)	Anaerobic (46)
10	5.8	9.3	8.9	12.7
20	9.5	18.8	17.3	23.4
30	14.3	32.7	24.5	38.2
40	19.7	—	37.3	—

Values in parentheses indicate the number of worms used in each experiment.

of that in glucose saline under both conditions (Table 1). However, the survival time of the parasite does not show any significant difference.

In experiments in which blood was not added to the medium the dark appearance of the fluke ceca disappeared after two or three days. Usually the dark pigment was regurgitated, but its quantity was less in worms kept in the blood free medium while greater in the blood containing medium. It appears that the worms feed on the bovine blood provided in the medium. Freshly prepared medium was adjusted to pH 7.0, but actively metabolizing worms lowered the pH of the medium to 5.5 to 4.5.

Certain subjective criteria were used to determine the well being of the worms during *in vitro* culture. The movement of the anterior region of the parasite was noticed with special attention and the mode

of attachment of the worms to the bottom of the culture flasks. Healthy and freshly cultured flukes showed rapidity in their wriggling movement. After a few days of living *in vitro* condition the flukes showed a gradual decrease in their size, with their movements slowed down. If no movement was noticed in any worm and colour of the parasite changed from red to white, it was considered to be dead. Besides, mature worms continued to lay eggs during the first ten days, after which the egg production deteriorated.

From the present *in vitro* experiments, it can be seen that not only does *I. hypselobagri* survive longer but the percentage survival is also higher under aerobic conditions than under anaerobic conditions. Under aerobic conditions the parasites remain more active and do not loose the colour of their tissue hemoglobin as rapidly as they do under anaerobic conditions. It can also be seen from the changes in the pH of the medium that the parasites are metabolically active during *in vitro* culture.

The exploratory *in vitro* experiments, carried out in simple glucose saline, show that if this parasite would be cultured in a more complete and chemically defined medium, the survival time could be enhanced and the weight loss counteracted. Even otherwise, *I. hypselobagri* survives 49 days under these simple conditions which is a much better record of survival compared with *Fasciola hepatica* (Rohrbacher, 1957) which survives 21–28 days in a balanced saline with liver extract; *Fascioloides magna* larva (Friedl, 1961a, b) which survives for 8–10 days in a medium containing amino acids. *Haematolechus medioplexus* (Churchill and Crowther, 1961) was kept alive for 40 days in Ringer's saline containing Difco nutrient agar. *Schistosoma mansoni* (Robinson, 1956) remains alive for 28–56 days in Tyrode with horse serum and glucose while Senft and Senft (1962) found that though *S. mansoni* survives for 20–45 days, after 20 days the egg production deteriorated when cultured in NCTC 109. This clearly indicates that *I. hypselobagri* which can survive up to 50 days in a simple glucose saline offers a unique opportunity to use it for *in vitro* culture studies. It can also prove to be an extremely useful model in the hands of parasite physiologists in elucidating many physiological aspects of trematodes in general. The saline medium containing only glucose and blood is obviously not ideal and does not meet the requirements of the trematode under investigation. It appears that a chemically defined medium should be devised in order to prolong *in vitro* survival, decrease the weight loss and increase the egg production of this parasite.

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Gas Content of Swim Bladder of *Wallago attu* and Oxygen Consumption in *Isoparorchis hypselobagri* (Trematoda)

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Summary. The swim bladder gas of *Wallago attu* contains O_2 (22–58 mm Hg) and the presence or absence of *Isoparorchis hypselobagri* does not influence the O_2 content of the swim bladder. Glucose increases the O_2 consumption of *I. hypselobagri* by 50%. With the passage of in vitro culture time, the rate of O_2 consumption gradually decreases to the extent of 64% on the 40th day. The optimal temperature for O_2 consumption is 30° C beyond which the rise in temperature is detrimental to the fish trematode as manifested by the decrease in their O_2 consumption.

Introduction

Though a number of adult digenetic trematodes have been studied for oxygen consumption (literature in von Brand, 1973), the influence of glucose and temperature on the oxygen consumption of trematodes living in oxygen rich environment has been studied in only a few species: *Paragonimus westermani*, *Schistosoma japonicum* (Shimomura, 1959); *Paragonimus ohirai* (Bruce *et al.*, 1971); *Schistosoma mansoni* (McNaughton, 1947; Bueding, 1950).

The purpose of this study was to determine the gas content of the swim bladder of the catfish and make exploratory studies on the influence of glucose, in vitro culture time and temperature on the rate of O_2 consumption in adult *Isoparorchis hypselobagri*. This trematode lives in the swim bladder of a catfish, *Wallago attu*, where O_2 is present and blood is the only source of nutrition.

Materials and Methods

The oxygen content of the swim bladder was determined by Krogh's micro-gas-analyzer, which is accurate to within 1–2% (Welsh and Smith, 1961). Living fishes were caught in the field and gas analysis was made on the spot by obtaining gas samples from the swim bladder. The gas samples were collected in 3 ml syringes over 1 ml of acidified saturated sodium chloride solution containing methyl red indicator (0.5% in 95% alcohol and a few drops of HCl). This solution is low in oxygen dissolving capacity. To avoid the contamination of the gas sample with air, the needle of the syringe was held upside down and was injected at the mid-lateral line, just posterior to the gill region about an inch deep into a freshly caught catfish. By gently moving the piston upward, about 2 ml of the swim bladder gas was taken up into the syringe, and small drops were introduced into the micro-gas-analyzer. "Oxsorbent" (Burrell Crop.) was used to absorb the oxygen from the sample. The results of the oxygen determinations were calculated as percentage of the total swim bladder gas and expressed as partial pressure of O_2 in mm Hg. The CO_2 was determined in a similar manner and 0.25 M KOH was used to absorb the gas. Nitrogen was calculated by subtracting the O_2 and CO_2 figures from the total gas content.

The oxygen consumption of the parasite was determined by Scholander's (1950) plastic microrespirometer, which was slightly modified and 15 ml Warburg flasks were used as animal chamber and the thermobarometer. The gas in this respirometer is kept under constant temperature and pressure and changes in gas volume are read directly by using an Agla

micrometer syringe (Welcome Laboratories, London). The preciseness and accuracy of the results obtained by this apparatus has been checked by Wennesland (1952), who had further modified this plastic microrespirometer and favourably compard his results with those obtained with standard Warburg manometry.

Active adult flukes were collected from the swim bladder of the catfish, and were allowed to stand for an hour in physiological saline which was adjusted at pH 7.5 to shed their eggs and hematin content of the intestinal ceca.

Two millilitres of glucose free or glucose containing saline (Nizami and Siddiqi, 1975) was placed in each flask and two to four flukes were placed in the animal chamber. Filter paper strip and 0.2 ml of 20% KOH were placed in the central well of the flask for the absorption of CO_2. Following the measurement of oxygen consumption, the worms were dried at 100° C for 24–36 hours, and the results were expressed as μl O_2 consumed/mg dry weight/hr. The oxygen consumption of the parasite in relation to in vitro culture time was also determined on the 10th, 20th, 30th and 40th day at 20° C. The metabolic temperature response in relation to O_2 consumption by the parasites was studied over a temperature of 20–45° C.

Results

The gas analysis of the swim bladder of *Wallago attu* showed that it contains a moderate quantity of oxygen, which ranges from 22.0 to 58.0 mm Hg or 2.9 to 7.9 volume percent while carbon dioxide ranged from 1.59 to 10.48 mm Hg or 0.21 to 1.38 volume percent of the swim bladder gas. Nitrogen is the major gas component in *Wallago attu*. It was also found that the presence or absence of the worms did not influence the O_2 or CO_2 content of the swim bladder gas. The results of the gas analysis are shown in Table 1.

Table 1. Gas content of swim bladder of *Wallago attu*. Values are in mmHg

No.	Oxygen	Carbon dioxide	Nitrogen[a]	*n*	Presence or absence of parasite
1	44.99	6.15	708.86	11	+
2	28.27	10.26	721.47	7	−
3	40.81	10.03	709.16	13	−
4	32.83	10.48	716.69	8	−
5	49.47	6.99	703.54	5	+
6	32.83	7.37	719.80	9	−
7	29.79	9.34	720.87	13	−
8	57.91	2.81	699.28	10	+
9	29.03	3.11	727.86	12	−
10	40.43	5.39	714.18	4	−
11	33.21	7.44	719.35	13	+
12	28.27	1.59	730.14	10	+
13	51.14	2.96	705.90	9	−
14	40.58	10.41	709.01	6	+
15	51.14	9.42	699.44	13	+
16	56.24	6.61	697.15	8	−
17	22.04	4.40	733.56	11	+
18	57.75	5.54	696.70	14	+
19	52.44	5.24	702.32	10	−
20	48.64	3.64	707.72	9	+

[a] Nitrogen has been calculated by deducting the oxygen and carbon dioxide contents from the total gas volume of the swim bladder.

n= No. of analyses.

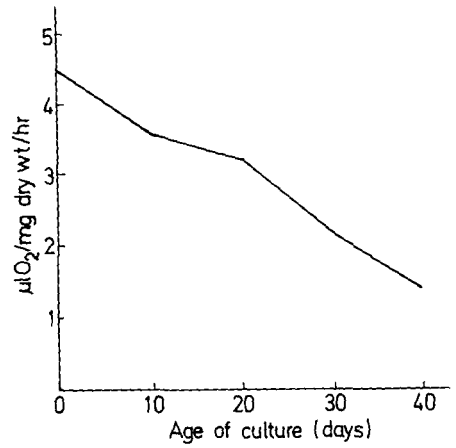

Fig. 1. Rate of oxygen consumption of *Isoparorchis hypselobagri* in relation to the age of in vitro culture

Table 2. Oxygen consumption of *I. hypselobagri* in µl O_2/mg dry weight in the absence and presence of glucose (8 mM)

Time (min)	No. of samples	Without glucose	With glucose	% Change
15	9	0.70	0.78	+11.6
30	11	1.80	2.3	+28.3
45	11	2.73	3.87	+41.7
60	13	3.03	4.54	+49.8

The results of the O_2 consumption by *I. hypselobagri* in the presence and absence of glucose are shown in Table 2. The exogenous glucose has marked influence on O_2 consumption and significantly increased the rate of O_2 utilization three to eight hours after collection of worms. Since *I. hypselobagri* survives well under aerobic conditions (Nizami and Siddiqi, 1975) the oxygen consumption of the worms in relation to *in vitro* culture time was also determined. With the passage of time, the rate of oxygen consumption of the worms decreases gradually on the 10th, 20th, 30th and 40th day by 16%, 23%, 51% and 64% respectively (Fig. 1).

The response of the fish trematode to temperature variation is quite pronounced. The oxygen consumption increases at 20° C to 30° C but at 35° to 45° C the oxygen consumption decreases. The rise in temperature beyond 35° C is extremely detrimental to the worms and the oxygen consumption of the worms decreases as shown in Fig. 2.

Discussion

Knowledge of the metabolism of trematodes under aerobic and anaerobic conditions and of their habitat is necessary for a better understanding of the respiratory physiology of these parasites. Many trematodes live in environments with high oxygen tension; for example, parasites of nasal passage, trachea, lungs

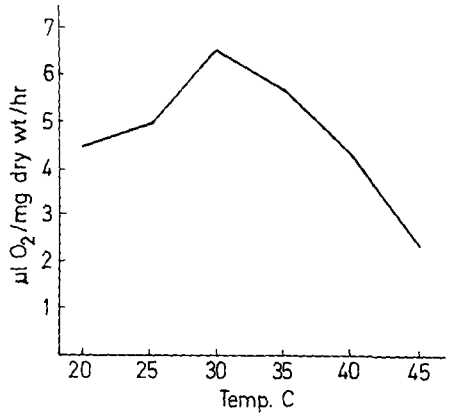

Fig. 2. The metabolic-temperature response of *Isoparorchis hypselobagri* at different temperatures

and arterial blood of various vertebrates and the swim bladder of fishes. The oxygen tension of various parasitic habitats have been tabulated by von Brand (1952). Although the data on the gaseous content of the swim bladder of fishes is controversial, it is certain that oxygen is a conspicuous gas in the swim bladder of physostomes (Hoar and Randall, 1970). Nitrogen obviously forms the major component and carbon dioxide only a minor component of the swim bladder gas in the present study. As can be seen from the results, the presence or absence of the trematodes in the swim bladder has no corelation with the contents of oxygen or carbon dioxide.

The swim bladder of *Wallago attu* is an oxygen rich environment, and the oxygen content is approximately half that of the oxygen content of arterial blood. It is, therefore, obvious that *I. hypselobagri* lives in a habitat which has biologically significant amounts of oxygen, and even if this O_2 is being utilized by the trematodes present in the swim bladder, the O_2 continues to be secreted into the swim bladder and the oxygen concentration is maintained within a certain range.

Adult helminths are generally considered to be facultative aerobes and use oxygen when available. *Schistosoma mansoni* could survive anaerobically but did better in the presence of oxygen (Bueding, 1949); *Fasciola hepatica* lost the color of its hemoglobin rapidly under anaerobic conditions (Rohrbacher, 1957). All parasites studied so far under aerobic conditions consume oxygen and produce carbon dioxide regardless of whether they lead a primarily aerobic or anaerobic life in their normal habitat.

The data available on oxygen consumption of trematode parasites indicate that the oxygen of the habitat of the parasite plays an important role. *Schistosoma mansoni* consumes more oxygen than those worms inhabiting areas of low oxygen tension, such as *Fasciola hepatica, F. gigantica, Dicrocoelium dendriticum; Echinostoma revolutum*, etc. According to Beitinger and Hammen (1971), as a general rule, it appears that parasitic nematodes have higher, the cestodes and acanthocephala intermediate, while the trematodes the lowest and most variable oxygen

uptake rate. *Isoparorchis hypselobagri* consumes oxygen and there is every likelihood that it might have an aerobic type of metabolism. The importance of oxygen consumption in an anaerobic organism is unknown, but one hypothesis relates this ability to an adaptation of their metabolism, while living in intermediate host or other hosts during earlier phase of their evolution (Beitinger and Hammen, 1971).

When glucose is added to the medium, the oxygen consumption was increased by 50%, which suggests that glucose is readily taken up and utilized as an energy source. This is in agreement with the findings of Bueding (1950) for *S. mansoni;* Vernberg and Hunter (1963) for *Himasthla quissetensis;* Pascoe (1968) for sporocyst of *Microphallus pygmaleus*, Taft and Fried (1968) for *E. revolutum* and Bruce *et al.* (1971) for *Paragonimus ohirai*. However, van Grembergen (1949) and Eckert and Lehner (1971) hold the opinion that glucose does not produce any stimulatory effect in the case of *Fasciola hepatica* and *Dicrocoelium dendriticum* respectively.

It has been reported that the culture forms of protozoa and cestodes show a declining rate of oxygen consumption with increasing age of culture, but the definite reason for this decline is still unknown (von Brand *et al.*, 1946). The decline in oxygen consumption of *I. hypselobagri* with the passage of time in vitro culture might be due to the fact that the constituents of the medium in which *I. hypselobagri* was kept were not optimal, and this resulted in gradual deterioration and consequent weight loss of the parasite. Contrary to the present results, Taft and Fried (1968) found that 8 to 29 days old specimens of *E. revolutum* had identical respiratory rates.

With respect to the effect of various temperatures on trematode respiration, it can be seen that the maximum oxygen uptake was observed at 30° C. Beyond 30° C, the rise in temperature adversely affects the O_2 uptake. The metabolic temperature response of *I. hypselobagri* is closely parallel to the temperature range which the fish host encounters. This agrees with the view of Vernberg and Hunter (1961), who hold the opinion that the response of parasites of poikilothermic and homoiothermic animals is closely parallel to the temperature range which each adult parasite encounters in its final host. The parasites from homoiothermic animals can survive higher temperatures better than those from poikilothermic hosts.

It would be interesting to examine the physiological and biochemical similarities and differences between the parasites of poikilothermic and homoiothermic animals, which might exist as a consequence of niche specialization. Rao and Bullock (1954) suggested that the temperature of the habitat influences the respiration of the free living animals. Vernberg and Hunter (1961) believe that this has also proved to be true for endoparasites.

Isoparorchis hypselobagri which lives in an oxygen rich environment in a poikilothermic animal and can be kept alive in vitro culture for long periods (Nizami and Siddiqi, 1975) provides an interesting opportunity for further studies on its respiratory metabolism.

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Non-specific alkaline phosphomonoesterases of eight species of digenetic trematodes

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ABSTRACT

Alkaline phosphatases from different trematodes occupying the same habitat have identical pH optima but different levels of enzyme activities. *Isoparorchis hypselobagri*, from the fish *Wallago attu*, shows four to six times more enzyme activity than *Fasciolopsis buski*, *Gastrodiscoides hominis* and *Echinostoma malayanum*, from the pig *Sus scrofa*, and *Fasciola gigantica*, *Gigantocotyle explanatum*, *Cotylophoron cotylophorum and Gastrothylax crumenifer*, from the buffalo *Bubalus bubalis*.

At least two peaks of activity at different levels of pH were obtained for each trematode examined. Both *Gastrodiscoides hominis* and *Isoparorchis hypselobagri* enzymes had three peaks of alkaline phosphatase activity.

The optimum temperature for maximum enzyme activity was 40°C, above which rapid inactivation occurred. At temperatures below 40°C, the enzymes of fish and mammalian trematodes did not behave similarly; *I. hypselobagri* enzyme being active over a wider range of temperature (20°–40°C).

Various concentrations of KCN and arsenate proportionately inhibited enzyme activity. NaF did not significantly influence enzyme activity, while Mg^{++} and Co^{++} acted as activators. The extent of inhibition or activation of enzyme activity of different trematodes varied, probably due to species differences. Both inhibition and activation of *I. hypselobagri* enzyme was higher than in the case of other trematodes.

The existence of non-specific phosphomonoesterases has been demonstrated in a number of trematodes. The majority of these investigations deal with the histochemical demonstration of phosphatases in parasite tissues and only a few are biochemical studies. The biochemical reports are confined mainly to the study of acid phosphatase; alkaline phosphatase having been studied in only a few trematodes.

The present investigation is an attempt to study some comparative aspects of the alkaline phosphatase systems in eight species of digenetic trematodes. One species from fish and seven different species of mammalian (cattle and pig) trematodes living in different habitats were studied for any interspecific differences that might exist as a result of living in various habitats in similar and different hosts.

MATERIALS AND METHODS

Fresh worms were collected from the local abattoir and fish market. *Gigantocotyle explanatum* and *Fasciola gigantica* were obtained from the liver and *Cotylophoron cotylophorum* and *Gastrothylax crumenifer* from the rumen, soon after the Indian water buffalo, *Bubalus bubalis L.*, was slaughtered. *Fasciolopsis buski*, and *Echinostoma malayanum* from the intestine, and *Gastrodiscoides hominis* from the caecum of pigs, were collected at the Central Dairy Farm, Aligarh. *Isoparorchis hypselobagri* were obtained from the swim bladder of the catfish, *Wallago attu*. The mammalian trematodes and fish trematodes were

washed several times in Tyrode saline and modified Ringer's saline (Forster and Taggart, 1950) respectively and were damp dried on a Whatman filter paper. The homogenates (10% w/v) of each species were prepared in a Potter-Elvehjem glass homogenizer in ice cold normal saline and centrifuged at 1,500 g for 30 minutes. The supernatant fluid was stored under toluene layer in the freezer. Storage in the freezer for up to two weeks did not result in enzyme inactivation. However, all experiments were carried out within 48 hours of enzyme extraction.

Assay of alkaline phosphatase

The non-specific phosphomonoesterase (orthophosphoric monoester phosphohydrolase, E.C. 3.1.3.1) activity was determined colorimetrically on a Hilger Biochem absorptiometer using filter No. 70 with appropriate blanks of buffered sodium β-glycerophosphate substrate as suggested by Hawk *et al.* (1954), and the liberated phosphorus was estimated by the method modified by Bartlett (1959). In a total volume of 10 ml, the reaction mixture contained: sodium β-glycerophosphate 142·3μ moles: sodium diethylbarbitone 184·4μ moles; and 0·5 ml of 10% (w/v) homogenate of parasites. The reaction was stopped with TCA after 60 minutes and phosphorus was estimated in protein free supernatant fluid at room temperature. Specific enzyme activity has been expressed in terms of μg inorganic phosphorus liberated per mg protein per hour. The protein concentration in homogenates was estimated by the method of Lowry *et al.* (1951) using crystalline bovine serum albumin as standard.

Effect of pH

The effect of pH on enzyme activity was studied using buffered (sodium diethyl barbitone) sodium β-glycerophosphate as substrate. Different pH levels ranging from 7·0–12·0 were adjusted using dilute acid and alkali.

Effect of temperature

The effect of temperature on trematode enzyme activity was studied at the following levels of pH: pH 8 for *I. hypselobagri*, pH 11 for *F. buski*, *G. hominis* and *E. malayanum*, pH 11·5 for *G. crumenifer* and *C. cotylophorum*, and pH 10 for *F. gigantica* and *G. explanatum*. The samples were incubated at five different temperatures ranging from 10°C–50°C for one hour and parallel control samples were maintained. At the end of the incubation period the enzyme activities were determined in the same manner as described above.

Effect of chemicals

The effect of certain chemicals on enzyme activity was also examined. KCN, Na_3AsO_4, $MgCl_2$ and $CoCl_2$ were used in the final concentrations shown in Table 2. These chemicals were added to the crude enzyme preparations just before enzyme activity was measured. The studies were made only at that pH at which the normal activity was found to be maximal.

All the experiments were carried out two to eight times and the points on the graphs represent the average of five readings.

RESULTS

Enzyme activity

From the results obtained in different experiments, it can be seen that alkaline phosphatase activity is present in all trematodes, though the relative enzyme activity was different in different species. In *I. hypselobagri*, the specific enzyme activity was four to six times higher than in other trematodes (Table 1).

(a) *Effect of pH;* Alkaline phosphatases in trematodes require an optimum alkaline environment for maximum enzymatic activity. In *I. hypselobagri* the maximum enzyme activities were observed at pH 8, 9 and 10–10·5 with maximum activity at pH 8 and 9. In the case of *F. buski* and *E. malayanum*, the maximum enzyme activity was observed at pH 7·5 and 11, and in *G. hominis* at pH 7·5, 9·5 and 11, with more pronounced activity at pH 7·5 and 11. In *F. gigantica* and *G. explanatum*, the activity was observed at pH 8 and 10, while *C. cotylophorum* and *G. crumenifer* showed maximum enzyme activity at pH 9 and 11·5. The results have been summarized in Table 1.

TABLE 1

Specific activities of alkaline phosphatases of trematodes at various pH optima.

pH optima are in parentheses; ± values are standard errors of the mean.

Species	Host	Location	*n*	Ist peak	IInd peak	IIIrd peak
I. hypselobagri	Fish	Swim bladder	8	95·23 ± 1·48 (8·0)	69·12 ± 1·13 (9·0)	39·16 ± 1·63 – 37·63 ± 1·85 (10 – 10·5)
F. buski	Pig	Intestine	5	23·45 ± 3·32 (7·5)	25·76 ± 2·85 (11·0)	—
E. malayanum	Pig	Intestine	5	13·14 ± 1·15 (7·5)	15·53 ± 0·95 (11·0)	—
G. hominis	Pig	Caecum	7	16·80 ± 2·35 (7·5)	12·70 ± 2·13 (9·5)	19·72 ± 2·70 (11·0)
F. gigantica	Buffalo	Liver	5	10·72 ± 0·85 (8·0)	20·24 ± 1·65 (10·0)	—
G. explanatum	Buffalo	Liver	8	12·35 ± 1·43 (8·0)	14·74 ± 2·12 (10·0)	—
C. cotylophorum	Buffalo	Rumen	7	8·20 ± 0·73 (9·0)	14·10 ± 0·97 (11·5)	—
G. crumenifer	Buffalo	Rumen	8	12·50 ± 1·17 (9·0)	18·31 ± 2·14 (11·5)	—

(b) *Effect of temperature;* The effect of temperature on the initial reaction velocity of trematode alkaline phosphatases was studied at five different temperatures ranging from 10°C–50°C (Figure 1). The enzyme is more susceptible to high temperatures and showed retardation in activity at low temperatures. In all trematodes except *I. hypselobagri* the enzyme activity was confined to a narrow range of 30°C–40°C. In the case of *I. hypselobagri* the enzyme was active over a wider range of temperature, from 20°C–40°C.

(c) *Effect of chemicals;* The results are given in Table 2.

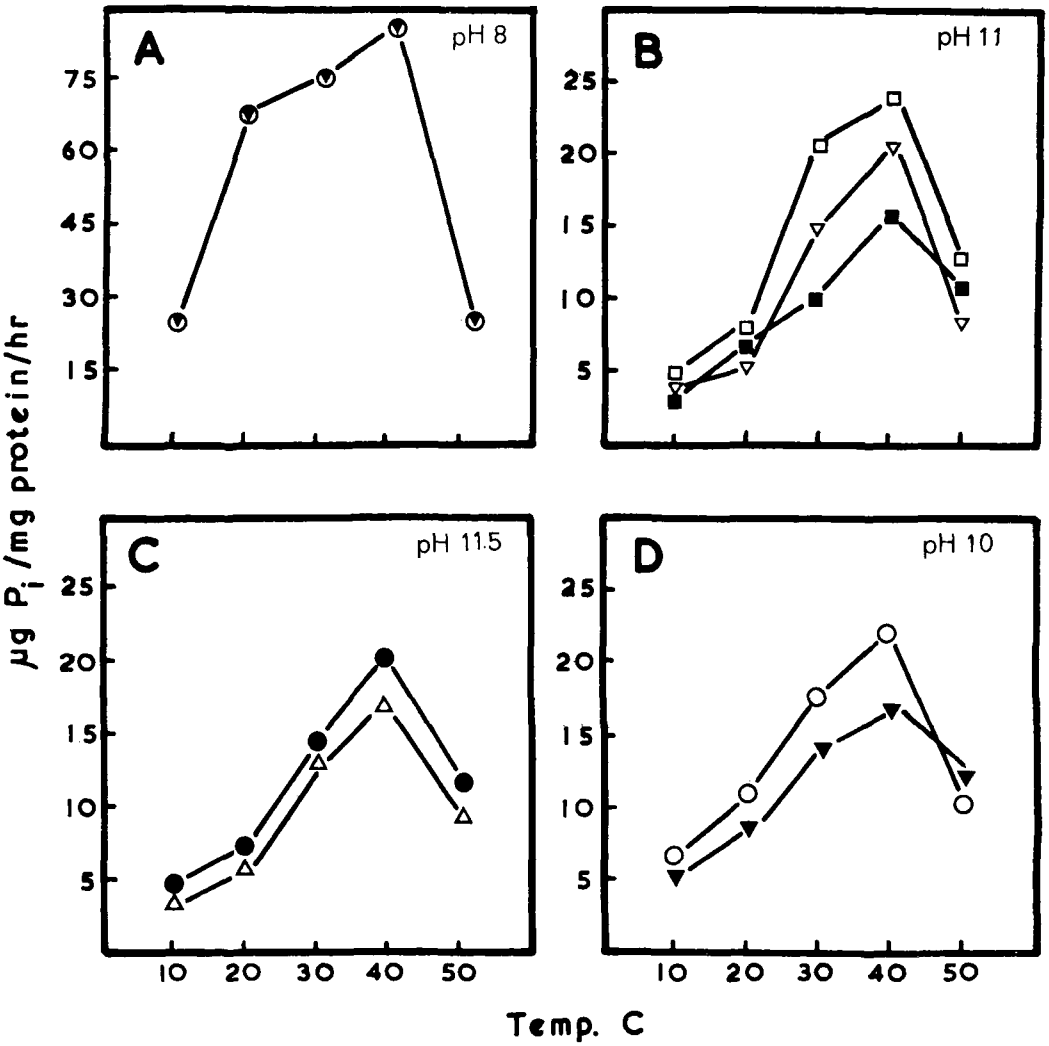

FIG. 1

Effect of temperature on alkaline phosphatase activity of trematodes. A—*Isoparorchis hypselobagri*; B—(□—□) *Fasciolopsis buski*; (▽—▽) *Gastrodiscoides hominis*; (■—■) *Echinostoma malayanum*; C—(●—●) *Gastrothylax crumenifer*; (△—△) *Cotylophoron cotylophorum*; D—(○—○) *Fasciola gigantica*; (▼—▼) *Gigantocotyle explanatum*.

TABLE 2

Effect of various chemicals on alkaline phosphatase activity of some trematodes.

Species	pH	KCN 10^{-2}M $n = 7$	KCN 10^{-3}M $n = 7$	KCN 10^{-4}M $n = 8$	Na_3AsO_4 10^{-3}M $n = 5$	Na_3AsO_4 10^{-4}M $n = 5$	NaF 10^{-2} $n = 7$	Mg^{++} 10^{-2}M $n = 8$	Co^{++} 10^{-3}M $n = 5$
I. hypselobagri	8·0	− 97·4	− 81·5	− 46·7	− 41·9	− 27·5	− 9·3	+ 21·9	+ 51·1
F. buski	11·0	− 93·3	− 87·3	− 26·8	− 28·3	− 12·1	− 1·3	+ 1·39	+ 22·4
G. hominis	11·0	− 89·2	− 82·4	− 23·5	− 21·0	−4·7	−0·9	+ 2·73	+ 39·0
E. malayanum	11·0	− 87·2	− 73·6	− 27·0	− 21·7	− 14·3	− 1·7	+ 3·1	+ 28·7
F. gigantica	10·0	−92·6	− 69·9	− 31·7	− 13·9	− 7·5	− 3·9	+ 4·78	+ 17·9
G. explanatum	10·0	− 89·3	− 70·3	− 42·1	− 19·3	− 11·7	− 0·8	+ 6·5	+ 23·1
C. cotylophorum	11·5	− 76·0	− 56·4	− 39·3	− 33·5	− 24·7	− 0·003	+ 4·9	+ 21·7
G. crumenifer	11·5	− 81·2	− 69·0	− 45·7	− 23·9	− 17·3	− 0·00	+ 7·1	+ 29·5

Values are percentage increase or decrease of control enzyme activity.

DISCUSSION

It can be seen from the results obtained in the present study that nonspecific alkaline phosphomonoesterases from different species of trematodes occupying similar habitats have identical pH optima but different relative enzyme specific activity. This activity seems to be species dependent and can be governed by many factors, including the age of the worms, state of metabolism, secretory state of the cells and individual adaptations on the part of the parasite to its habitat, etc. Of course, the value of Km could also be helpful in understanding the variations in the relative enzyme activities, but because all the experiments were carried out under optimal assay conditions, the differences in enzyme specific activities between various species of trematodes due to any other factor can safely be excluded.

It is also interesting to note that the pH optima for alkaline phosphatase activity in the case of rumen trematodes of buffalo, *C. cotylophorum* and *G. crumenifer* are identical. Similar is the case with the trematodes of the intestine of pig (*F. buski* and *E. malayanum*) and liver flukes of buffalo (*G. explanatum* and *F. gigantica*). The similarity in pH optima between different species occupying a similar habitat is a reflection of niche specialization. On the other hand, *G. hominis* from caecum of pig showed enzyme activities at 3 pH optima, two similar to, and one different from the pH optima of enzymes of *F. buski* and *E. malayanum*. However, when pH optima of the enzyme activities of trematodes from intestine of pig are compared with the pH optima of enzyme activities of trematodes from rumen, liver, caecum or swim bladder, differences are seen, and these may be a reflection of the differences in the habitats.

In each parasite there is more than one peak of enzyme activity at different levels of pH, maximum being three in *G. hominis* and *I. hypselobagri* which clearly means that alkaline phosphatases in trematodes probably originate from various subcellular organelles. Also according to von Brand (1973) "acid and alkaline" phosphatases are group names and more than one such enzyme can be found in an organism. The occurrence of more than one peak is suggestive of more than one enzyme.

The maximum activity in each species was observed at an optimum temperature of 40°C (Figure 1). Beyond 40°C the rise in temperature adversely effects the enzyme activity in all cases. Alkaline phosphatase of the fish trematodes appears to be different from enzymes of all other species under study, and shows gradual increase in activity over a wider range of temperature from 20°C–40°C, whereas in the case of mammalian trematodes the maximum enzyme activity peaks are seen at 40°C and on either side of this temperature the activity decreases quickly. The only other study on the effect of temperature on alkaline phosphatase activity is by Probert and Lwin (1974), who have shown that though acid phosphatase remained active up to 60°C, alkaline phosphatase activity was maximum at 37°C. Our results support and confirm this finding.

Probably these differences are due to the fact that trematodes from cattle and pig live in homeothermic animals, whereas *I. hypselobagri* lives in a poikilothermic animal. The latter is subject to many temperature variations. It would be interesting to examine and study the physiological and biochemical similarities and differences between these trematodes which might exist as a result of niche specialization. This fact can be verified only by carrying out enzyme studies on other trematodes living in poikilothermic animals.

As shown in Table 2, various chemical compounds have different effects on alkaline phosphatase activity. Different concentrations of KCN caused proportionately marked inhibition of the alkaline phosphatase activity and 10^{-2}M KCN almost completely inactivated the enzyme of *I. hypselobagri*, while the same concentration of KCN caused only 76% inhibition in *C. cotylophorum*. However, the enzymes from pig trematodes appeared to be less sensitive to low concentrations of KCN. Similar species differences in the extent of inhibition have also been reported in the case of alkaline phosphatase enzymes of other trematodes (Halton, 1967; Probert, *et al.*, 1972; Probert and Lwin, 1974). Like KCN, arsenate was also found to be more inhibitory for the enzymes of *I. hypselobagri*, than for enzymes from cattle and pig trematodes. Sodium fluoride proved to be a poor inhibitor even at a concentration of 10^{-2}M and in most species the decrease in enzyme activity is not more than 1–4% except in the case of *I. hypselobagri*, where also the inhibition is only 9·3%.

Both Mg^{++} and Co^{++} ions activate the enzyme although the latter is better. Magnesium at a concentration of 10^{-2}M does not significantly activate the enzymes from mammalian trematodes, although this concentration was enough to activate the alkaline phosphatase of *I. hypselobagri*. An explanation for this activation of fish trematode enzyme is not clear but it may be that Mg^{++} forms a component of this enzyme as has been suggested for other phosphatases (Roche, 1950). A positive activation of alkaline phosphatases in all trematodes under study was achieved with Co^{++}, although the maximum activation (50%) was seen again in the case of *I. hypselobagri*, whereas in all other trematodes the cobalt activation was much less (20–39%). It can be seen that, on the whole, the alkaline phosphatase from *I. hypselobagri* is more susceptible to the various inhibitors used and the degree of inhibition was almost always higher than in the case of enzymes of other species under study. Similarly, though Mg^{++} and Co^{++} act as activators of trematode alkaline phosphatases, the degree of activation is much higher in *I. hypselobagri* (21·9% under Mg^{++} and 51% under Co^{++}) than in other species (Table 2). This reflects an individual sensitivity on the part of a trematode enzyme towards different chemicals.

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OSMOTIC AND IONIC BEHAVIOUR OF SOME DIGENETIC TREMATODES

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Abstract—1. Osmotic and ionic behaviour of some digenetic trematodes—*Cotylophoron cotylophorum*, *Gastrothylax crumenifer* and *Gigantocotyle explanatum* from the buffalo, *Bubalus bubalis*, and *Isoparorchis hypselobagri* from the fish, *Wallago attu*—were studied in normal, diluted and concentrated salines and were found to be poikilosmotic.

2. In all species, weight gain is quicker than weight loss and permeability to water is greater than to salts, except in *Isoparorchis*, which does not strictly follow these laws of osmosis. It is more sensitive to hyperosmotic than to hypo-osmotic salines.

3. All species behave as leaky osmometers and lose Na^+ and K^+ by simple diffusion except *Isoparorchis*, in which Na^+ and K^+ are not lost to the same extent, especially in deionized water.

4. Differences in the osmotic and ionic behaviour of trematodes are due to their water and/or ionic content. They may also be due to the differential permeability of their teguments which appear to be a consequence of parasitism in different habitats.

INTRODUCTION

THE STUDY of osmotic and ionic behaviour in helminths has been confined only to the nematodes and cestodes, and trematodes have remained somewhat neglected. There are some scattered studies on the latter group and for complete references the reader is referred to Siddiqi & Lutz (1966), which is a detailed study of osmotic and ionic behaviour in a digenetic trematode, *Fasciola gigantica*. More recently, Bair and Peters (1971) found weight loss and oxygen consumption as valid parameters of osmotic activity in *Haematolechus medioplexus*. However, their data on O_2 consumption of *Haematolechus* does not support their claim since differences in O_2 consumption in worms subjected to various concentrations of NaCl do not seem to be significant. Until a number of trematode species from different hosts and habitats are studied, a decided gap exists in our understanding of this aspect of trematode physiology.

The present investigation is an attempt to study some comparative aspects of the osmotic and ionic behaviour of trematodes. For this purpose, four different species of trematodes were chosen. They were: *Cotylophoron cotylophorum* (Fischoeder, 1901); *Gastrothylax crumenifer* (Crepl, 1847) both from the rumen and *Gigantocotyle explanatum* (Crepl, 1847) from the bile ducts of buffalo, *Bubalus bubalis*, and *Isoparorchis hypselobagri* (Billet, 1898), from the swim bladder of the catfish, *Wallago attu*. The choice of these four species was considered important since they parasitize different habitats in two different species of vertebrate hosts and offer an interesting opportunity to examine, on a comparative basis, the differences, if any, in their osmotic and ionic behaviour.

MATERIALS AND METHODS

Fresh trematodes were obtained from the local abbatoir and fish market. *Cotylophoron cotylophorum* and *Gastrothylax crumenifer* were collected from the rumen and *Gigantocotyle explanatum* from the liver, soon after the buffaloes were slaughtered. The worms were transferred to Tyrode solution (NaCl 136 mM, KCl 2·6 mM, $CaCl_2$ 1·8 mM, $NaHCO_3$ 1·1 mM, NaH_2PO_4 0·32 mM and $MgCl_2$ 0·9 mM), and were incubated in this medium in a water-bath kept at 37± 1°C. For *Isoparorchis hypselobagri*, the swim bladders of *Wallago attu* were brought to the laboratory and the worms were collected and quickly rinsed in Ringer's as modified by Forster & Taggart (1950) for fresh water fish and containing NaCl 100 mM, KCl 2·5 mM, $CaCl_2$ 1·5 mM, $MgCl_2$ 1·0 mM, NaH_2PO_4 0·5 mM, $NaHCO_3$ 5 mM, and were incubated in the same medium in a water-bath maintained at 25 ± 1°C.

All experiments were begun soon after the worms were removed from their respective habitats and in all cases only mature worms were subjected to various dilutions of the two salines and deionized water for both osmotic and ionic studies. Normal Tyrode and Ringer were presumed to be isosmotic to the cattle and fish trematodes respectively.

For weight change studies and ionic concentration and regulation the methods used were essentially those described earlier by Siddiqi & Lutz (1966). In most experiments at least ten to fifteen worms were used. Weight change studies were made on a single pan balance Owa Labor Model 704 and sodium and potassium were

determined with a Systronic Flame Photometer, Model 121.

RESULTS

Osmotic behaviour in different media

To obtain a preliminary idea about the osmotic behaviour of the four species of trematodes, and to determine the isotonicity of the media, the worms were first subjected for a fixed period of 1 hr, to normal, diluted and concentrated Tyrode and Ringer solutions. The results are shown in Fig. 1. The four

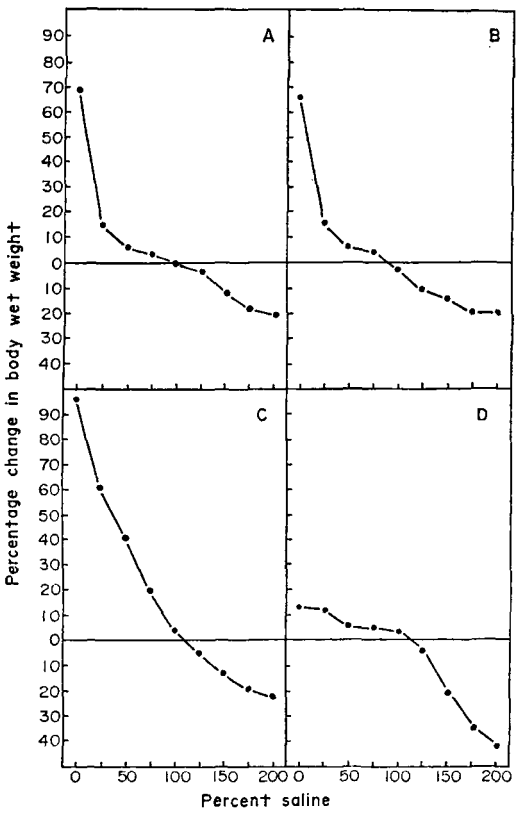

Fig. 1. Percentage change in body wet weight of trematodes after 1 hr in various concentrations of salines. A.—*C. cotylophorum*; B.—*G. crumenifer*; C.—*G. explanatum*; D.—*I. hypselobagri.*

species do not respond to various concentrations of salines in a similar manner. The rumen trematodes, *Cotylophoron* and *Gastrothylax*, show pronounced weight changes between 0 and 25% Tyrode and thereafter the weight changes are not exactly in direct relation to concentration of the surrounding media. On the other hand, in the liver fluke, *Gigantocotyle*, the body weight change is in direct relation to the various concentrations of the incubating medium; whereas the swim bladder trematode, *Isoparorchis*, is not sensitive to hypo-osmotic solutions and gains no more than 15 per cent of its body weight. The behaviour of *Cotylophoron*, *Gastrothylax* and *Gigantocotyle* is similar in hyperosmotic solutions and the weight loss is in the range of 20–25 per cent. However, *Isoparorchis* is very sensitive to hyperosmotic solutions and loses weight in proportion to the concentration of the surrounding media; the weight loss being 40 in 200% saline, which is nearly twice that of the other three species.

It can also be seen from these results that the normal salines used for these trematodes are not entirely isosmotic except in the case of *Cotylophoron*, which does not show any weight change in 100% Tyrode.

Tolerance of hypo-osmotic and hyperosmotic media

In another set of experiments the osmotic response of the four species was studied as a function of time in different dilutions of salines. The results are shown in Fig. 2.

What has been said above about the osmotic behaviour of the four species of trematodes, holds good and is supported by the present set of experiments. It can be seen from the results that *Gigantocotyle* is quite sensitive to 0 and 25% Tyrode and comes to equilibrium in a short period of time in 50 and 75% Tyrode. The overall response to hypo-osmotic and hyperosmotic salinities is the same as reported for *F. gigantica*. The rumen trematodes, *Cotylophoron* and *Gastrothylax*, show more or less an identical response but are sensitive only to deionized water. In 25, 50 and 75% and in the hyperosmotic media these worms adjust quickly and the weight changes are not as pronounced as in *Gigantocotyle*.

Isoparorchis behaves quite differently from the other three species under study. In this case the weight change is not more than 20–30 per cent in hypo-osmotic salines and the adjustment to all salinities, including the deionized water, takes place in a short period of time. However, *Isoparorchis* is more sensitive to higher salinities. The weight change is more in this case in all hyperosmotic solutions than in the case of the other three species.

Effect of hypo-osmotic and hyperosmotic media on survival

The effect of various concentrations of the physiological salines on the survival of the four species is also not identical. *Cotylophoron* and *Gastrothylax* are sensitive only to deionized water and survive in 25 per cent, though the change in body volume is pronounced (40 per cent of their original weight). *Gigantocotyle* becomes water-logged in deionized water and 25% Tyrode and dies in a short period of time; the weight increase is nearly 70–100 per cent. *Isoparorchis* does not suffer

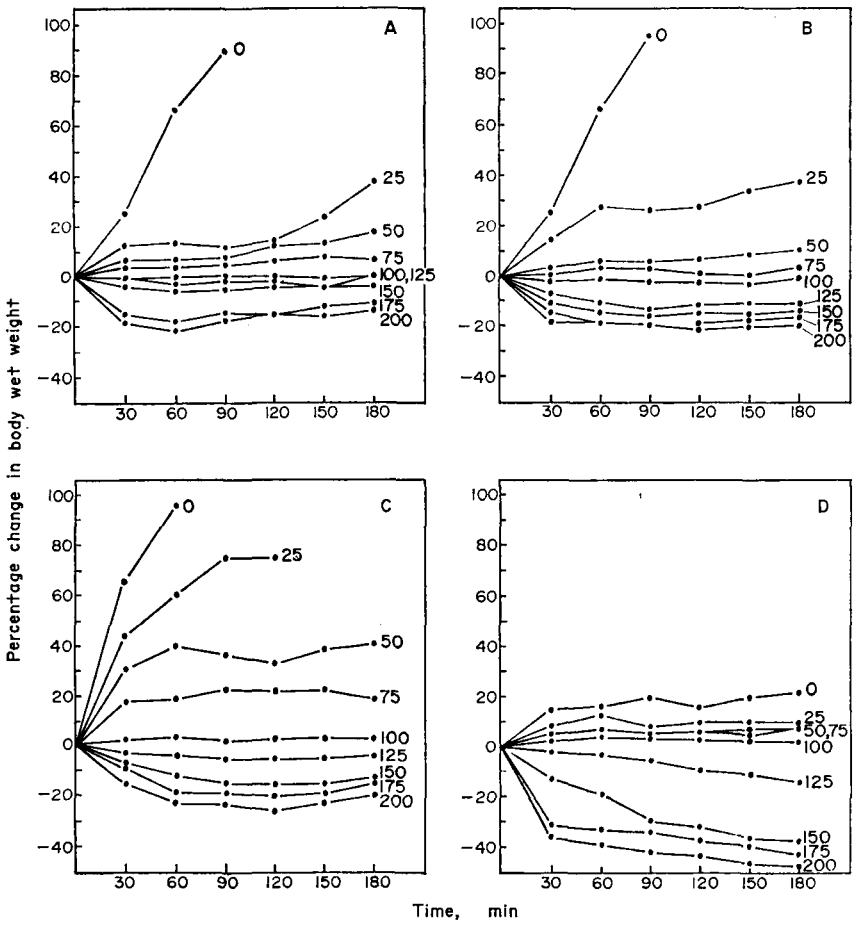

Fig. 2. Percentage change in body wet weight of trematodes when transferred at zero time to various concentrations of salines. Figures at the end of each line in the graphs indicate the concentration of salines. A.—*C. cotylophorum*; B.—*G. crumenifer*; C.—*G. explanatum*; D.—*I. hypselobagri.*

any ill effects and survives for long periods in both deionized water and 25% Ringer. As a matter of fact these worms stay in an active state of well being for long periods in low salinities.

Weight changes on transference to different media

In view of the fact that the four species under study behave differently in their osmotic behaviour, it was decided to subject them to transfer studies so as to examine their ability to readjust in normal salines after having been exposed to hypo- and hyperosmotic salines. The results are shown in Fig. 3. In these experiments, two sets of worms were preadapted to 75 and 125% salines for 1 hr and then transferred to 100% salines at zero time and their behaviour was noted. *Cotylophoron*, *Gastrothylax* and *Gigantocotyle* more or less regain their original body weight in 15–45 min followed by a slight increase and a decrease in weight of worms when transferred

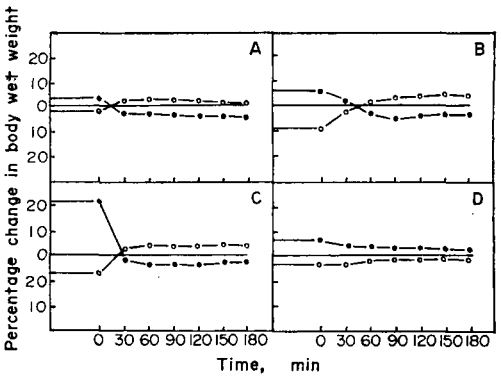

Fig. 3. Percentage change in body wet weight of two sets of trematodes preadapted to 75 and 125% salines for 1 hr and then transferred at zero time to normal salines: ●——●, 75%; ○——○, 125%. A.—*C. cotylophorum*; B.—*G. crumenifer*; C.—*G. explanatum*; D.—*I. hypselobagri.*

from hyperosmotic and hypo-osmotic media to normal salines respectively.

In *Isoparorchis* the percentage change in body weight in 125 and 75% Ringer is not significant to begin with (less than 10 per cent). As a result the original weight is not regained for several hours, and there is neither an increase nor a decrease in body weights in either set of worms when transferred from hyper- or hypo-osmotic salines to normal salines. In this respect *Isoparorchis* behaves in a different manner from the other three species.

Ionic concentration and regulation

Fresh worms were analysed for their total sodium and potassium content. The results are given in Table 1.

Table 1. Normal content of Na^+ and K^+ in trematodes in mM/kg of initial wet wt

Species	Habitat	Na^+	K^+	Na^+/K^+ ratio
Cotylophoron	Rumen	63·46	40·38	1·5
Gastrothylax	Rumen	69·40	48·92	1·4
Gigantocotyle	Liver	42·86	37·51	1·1
Isoparorchis	Swim bladder	35·91	33·17	1·05

Normal sodium and potassium values and their proportion to each other in *Cotylophoron* and *Gastrothylax* are of the same order in both species; the Na^+/K^+ ratio being approximately 1·4–1·5; whereas in *Gigantocotyle* and *Isoparorchis* the Na^+ and K^+ values are lower than those of rumen trematodes and the Na^+ content is more or less equal to the K^+ content; the Na^+/K^+ ratio being approximately 1.

For the determination of ionic loss and retention a number of worms of all four species were subjected to various dilutions for 180 min and their sodium and potassium content was determined at the end of each experiment. The sodium and potassium loss was calculated on the basis of total ionic content of fresh worms. The results are shown in Fig. 4.

Sodium is lost in all species in all dilutions in proportion to the surrounding medium up to 25%. *Isoparorchis* and *Gastrothylax*, however, lose less Na^+ in deionized water when compared with the other two species. They are able to retain up to 40 per cent of their Na^+ when the other two species are able to retain only 20 per cent of their Na^+ at the end of 180 min.

Potassium is also lost in all species in all dilutions in proportion to the surrounding media except in the case of *Isoparorchis* and *Gastrothylax* in which the loss is less compared with the other species. However, *Isoparorchis* retains up to 50 per cent of K^+ in deionized water when the other three species are able to retain only 20–30 per cent of their K^+ content. In other words, the K^+ loss in *Isoparorchis* is least among the four species.

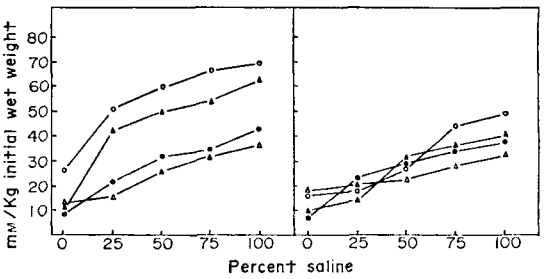

Fig. 4. Total body Na^+ and K^+ of trematodes, calculated on initial wet weight basis after 180 min in various concentrations of salines. ▲——▲, *C. cotylophorum*; ○——○, *G. crumenifer*; ●——●, *G. explanatum*; △——△, *I. hypselobagri.*

Ionic flux in deionized water

All four species were incubated in deionized water for 180 min and their sodium and potassium content was determined at suitable intervals. The deionized water was changed frequently. The results are shown in Fig. 5 and the percentage loss of Na^+ and K^+ in deionized water is shown in Tables 2 and 3. It can be seen that all three species of trematodes from the buffalo lose approximately 50 per cent of their ionic content in the first 30 min, whereas *Isoparorchis* loses only 15–18 per cent of its ionic content in the

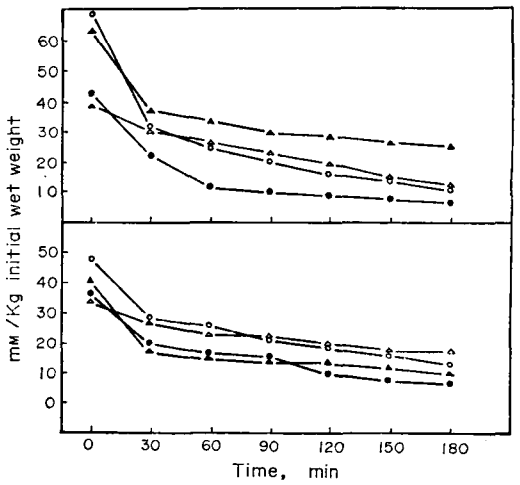

Fig. 5. Ionic flux in deionized water. Net loss of Na^+ and K^+ in trematodes calculated on initial wet weight basis in deionized water at various intervals. ▲——▲, *C. cotylophorum*; ○——○, *G. crumenifer*; ●——●, *G. explanatum*; △——△, *I. hypselobagri.*

same period of time. This ability to retain Na^+ and K^+ is maintained until the end of the experiment. *Isoparorchis* retains nearly 40–56 per cent of its ionic content when the other three species are able to retain only 18–45% Na^+ and K^+.

Table 2. Ionic flux in deionized water. Percentage loss of Na^+

Species	Time (min)					
	30	60	90	120	150	180
Cotylophoron	48·2	60·5	67·4	74·3	77·7	82·1
Gastrothylax	53·5	53·8	56·8	58·4	61·6	64·5
Gigantocotyle	46·5	68·5	73·6	77·2	80·9	82·8
Isoparorchis	14·5	24·0	33·5	43·8	57·2	58·8

Table 3. Ionic flux in deionized water. Percentage loss of K^+

Species	Time (min)					
	30	60	90	120	150	180
Cotylophoron	50·9	59·0	63·1	66·8	69·8	74·7
Gastrothylax	40·3	46·1	53·4	58·9	68·8	73·9
Gigantocotyle	45·6	53·4	57·6	69·9	75·0	77·9
Isoparorchis	17·6	27·8	27·8	40·2	42·8	44·7

DISCUSSION

The results of the present study support and extend most of the findings of Siddiqi & Lutz (1966) on the osmotic and ionic behaviour in *F. gigantica*. Though there is no doubt that trematodes are poikilosmotic invertebrates and behave like leaky osmometers, there are specific differences as far as their osmotic and ionic behaviour is concerned. From Fig. 1 it can be seen that, with the exception of *Cotylophoron*, the other three species do not appear to be completely isotonic to the normal salines used for them. However, the weight change is not more than ±3–4 per cent in any trematode species under study.

It is suggested that freezing point depression (Δ^0) of trematode tissues and Tyrode–Ringer solutions should be determined and the salines should be ionically modified according to the Δ^0 of the trematodes for future studies. The rumen trematodes *Cotylophoron* and *Gastrothylax* behave identically in various dilutions and the weight change is more pronounced only in deionized water. The behaviour of the liver fluke, *Gigantocotyle*, is exactly like *F. gigantica* and a simple relationship exists between weight change and the concentration of the surrounding media.

Isoparorchis behaves quite differently from the other three species. The extent of weight change in hypo-osmotic solutions is much less as can be seen in Fig. 1. However, it loses more weight in hyperosmotic media than the other three species.

From the results of the second set of experiments, in which weight change studies have been made in different dilutions of salines over an extended period of time, it can be seen that deionized water causes tremendous weight gain (over 70–90 per cent) in all species in the first hour except in the case of *Isoparorchis*. Other species become water-logged and die in a short period of time; whereas *Isoparorchis* does not gain more than 15 per cent of its weight and survives till the end of the experiment.

The behaviour pattern of *Cotylophoron* and *Gastrothylax* is more or less identical in all concentrations. They reach a new weight equilibrium in all salinities above 25 per cent and maintain it until the end of the experiments. They also tolerate the hypertonicities quite well.

Isoparorchis appears to be more sensitive to hyperosmotic solutions than the other species. To some extent, it loses weight in proportion to the concentration of the surrounding media; the higher the concentration, the greater is the weight loss. This behaviour of *Isoparorchis* is probably due to a greater content of water, which is lost by exosmosis in higher salinities and results in a pronounced weight loss. To verify this fact, wet and dry weights were determined and it was found that the water content of *Isoparorchis* is 92 per cent of the total wet weight; whereas in the other three species the water content averages from 68 to 80 per cent of the total wet weight.

From the results of these experiments it can be concluded that not only the weight gain is quicker than weight loss but the extent of weight gain is higher than the extent of weight loss in all species except *Isoparorchis*. In other words, the weight changes are in accordance with the laws of osmosis in the case of all species except *Isoparorchis*.

Higher tolerance of hypo-osmotic solutions and deionized water on the part of *Isoparorchis* does not appear to be a consequence of osmoregulation. The ionic content of this trematode is low and the water content is high; therefore, the worm does not tend to gain weight in hypo-osmotic media, or it probably possesses a tegument which is less permeable than in the case of the other three species. Differential permeability is probably an important factor and this is certainly under the influence of the habitat of parasites, and differs in different species. Those animals which live in fresh water, and can osmoregulate, are less permeable; brackish water crustaceans are less permeable than marine ones as pointed out by Potts & Parry (1963). In other words the higher the tonicity of the habitat the higher the permeability of the tegument.

Siddiqi & Lutz (1966) pointed out that the flat worms behave like marine invertebrates and their

permeability to salt and water is of a high degree. The differences in the osmotic behaviour of trematodes in the present study are merely due to differences in their habitats. The rumen flukes, *Cotylophoron* and *Gastrothylax*, are subjected to frequent changes of the surrounding medium in their natural environment and thus seem to be better adapted to lower tonicities as can be seen from their response to 25% Tyrode. *Gigantocotyle*, being a parasite of the liver bile duct, is very sensitive to deionized water and 25% Tyrode and does not tolerate either, since it lives in a more or less osmotically constant environment, as is also true of *F. gigantica*.

Isoparorchis lives in an environment where there is no exchange of fluids. Food is obtained by feeding on the blood of the host and no nutrients are present in the immediate vicinity of the worms. This feature of the habitat has probably influenced the permeability of the tegument of the worm. Since no function appears to be performed by the tegument, the latter would be of a different nature than in the case of other trematodes. This can be verified only after the ultrastructure of the tegument has been studied by electron microscopy. In general, the overall situation in trematodes appears to be more or less similar to cestodes in which osmotic concentration and regulation remains closely parallel to that of the host's habitat, i.e. the intestinal tract (Smyth, 1946).

The transfer studies show that *Cotylophoron*, *Gastrothylax* and *Gigantocotyle*, when transferred to 100% salines after having been preadapted to hypo-osmotic and hyperosmotic media, not only regain their original weights but the set preadapted to 75% Tyrode lose weight and the one preadapted to 125% Tyrode gains weight. This phenomenon was also observed but not explained by Siddiqi & Lutz (1966) in the case of *F. gigantica*. From the present results it can be concluded that the worms exposed to either hypo-osmotic or hyperosmotic media adjust to their new surroundings by water osmosis and salt loss or gain and become isotonic to their changed environment. When these preadapted worms are transferred to 100% Tyrode, the latter acts as a hyperosmotic medium for the 75% set and hypo-osmotic medium for the 125% set. The result is weight loss in the former case and weight gain in the latter. The passage of water into the worms and outward flux of salts appears to be phasic also in these trematodes as was noticed by Webster (1970) in *Hymenolepis diminuta*. From these results it can also be concluded that permeability to water is greater than permeability to salts.

Isoparorchis, however, does not follow this law of osmosis. Either set of worms preadapted to 75 or 125% Ringer does not show significant weight changes, and the worms do not regain their original weight for several hours. These results also support the view that *Isoparorchis* tegument is less permeable than is the case in the other three species.

Ionic concentration and regulation

Our knowledge of the normal sodium and potassium content of trematodes is limited. The present values in Table 1 compare favourably with those reported for *F. gigantica* by Siddiqi & Lutz (1966). Species differences, however, exist among the four species studied. *Isoparorchis* and *Gigantocotyle* possess Na^+ and K^+ in almost equal amounts and in this respect they are different from *Cotylophoron* and *Gastrothylax*. The Na^+/K^+ ratio is also very low (1–1·5) in all the four species studied.

Siddiqi & Lutz (1966) reported some difference in Na^+ and K^+ loss in *F. gigantica*. They found that K^+ is maintained to a dilution of 75 per cent and Na^+ is lost in all hypo-osmotic dilutions. In the present study no such difference was observed. All four species lose Na^+ and K^+ in proportion to the concentration of the surrounding medium except in the case of deionized water, in which individual differences in ionic loss and retention are seen. *Isoparorchis* appears to behave quite differently from the other three species in Na^+ and K^+ retention as it also does in its osmotic behaviour.

Ionic flux in deionized water

Ionic loss is pronounced in deionized water in *Cotylophoron*, *Gastrothylax* and *Gigantocotyle* and nearly 50 per cent of their total ionic content is lost in the first 30 min. Thereafter the rate declines and only 25 per cent of their total ionic content is lost in the next 2·5 hr, whereas in the case of *Isoparorchis*, there is a minimum loss of only 17·6% K^+ and 14·5% Na^+ in the first 30 min and the total loss in the next 2·5 hr is not more than 45–59 per cent of the total ionic content. Also at different time intervals, the rate of Na^+ and K^+ loss is much less in *Isoparorchis* than in the other species (Tables 2 and 3). It therefore appears that when *Cotylophoron*, *Gastrothylax* and *Gigantocotyle* have lost most of their ionic content (60–80%), *Isoparorchis* loses only 45–49 per cent of its Na^+ and K^+. This becomes all the more interesting when one also keeps in mind the fact that the normal Na^+ and K^+ content of this species is lower to that of the other three species.

This could be either due to active ionic retention or decreased permeability of the tegument as pointed out earlier. The latter is most likely the case, since trematodes are osmo-conformers and they have lost all ability to osmoregulate. As in the case of other trematodes so far studied, the present investigation also supports the fact that these worms behave as leaky osmometers, and lose salts by simple diffusion. However, the degree or extent of ionic loss varies with different species.

SUMMARY

All the four species studied in the present investigation adjust osmotically and ionically as a result of rapid water influx and outward ionic flux by simple diffusion when subjected to various dilutions of Tyrode and Ringer solutions. Though they are poikilosmotic, they show specific differences in their tolerance to various dilutions of the salines used. Either the ionic or water content and/or the trematode tegument appears to be responsible for the differences in their osmotic and ionic behaviour. Though no evidence of partial active osmotic or ionic regulation is available, it is obvious that trematodes appear to possess tegument with different permeability, which may be a result of individual parasitic adaptation to different habitats. In brief the role of the habitat appears to be very important in the physiology of trematodes, and the nature of the host habitat, to a large extent, determines the permeability of the trematode tegument to water and salts. The ultrastructure of the tegument of all the four species should be studied by electron microscopy to verify this conjecture.

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